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REGULAR DEPARTMENTS

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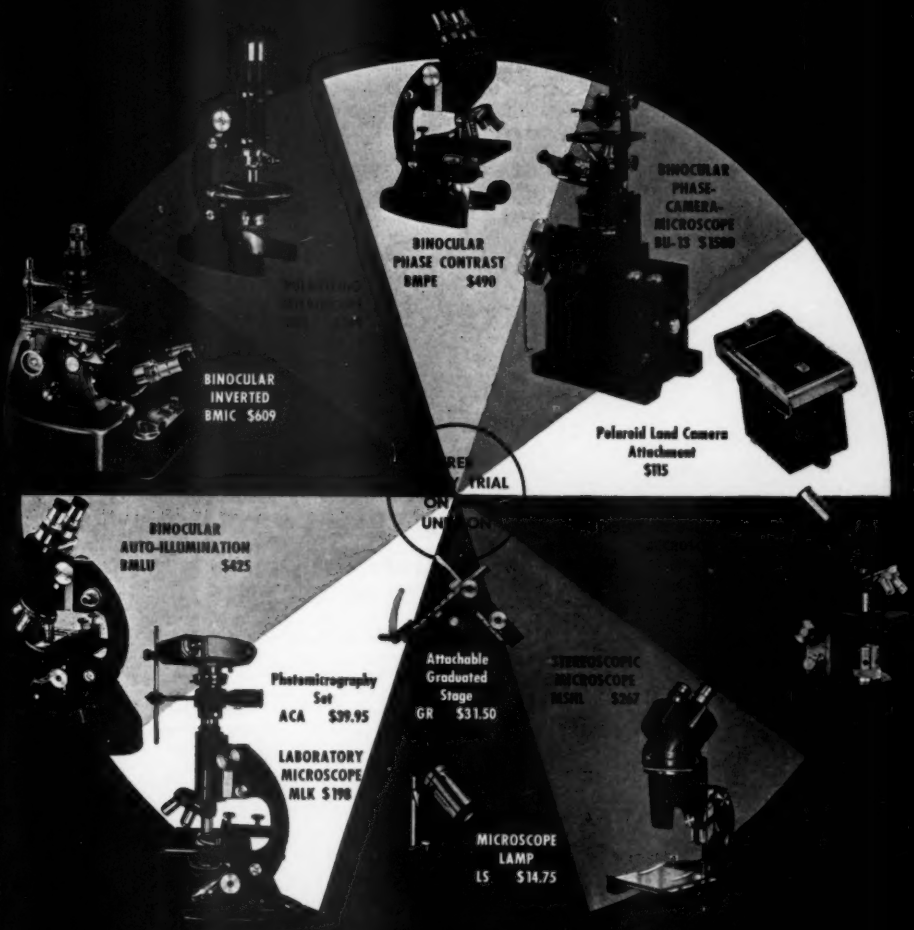

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A.M.A. ARCHIVES OF
PATHOLOGY

Masters of Modern Pathology: Jakob Erdheim

S. M. RABSON, M.D., Los Angeles

An examination of Erdheim's bibliography makes clear the chief fields of his labors—skeleton and organs of internal secretion, from aberrations of which he himself suffered. The papers include those now considered landmarks: hypophyseal changes during pregnancy, hypophyseal-duct neoplasia (the "Erdheim tumor"), the role of the parathyroid glands in calcium metabolism and dentition, secondary parathyroidal hypertrophy in rickets and osteomalacia, osseous changes in acromegaly and the relation of eosinophilic adenomas to that disturbance, and aortic cystic medial necrosis. The bibliography, however, is misleadingly short. Many contributions are of great length, some totaling well over 100 pages, and treat several aspects of the given problem. In addition, for every publication bearing his name, there are a half-dozen ascribed to his pupils, the titular authors, who were, however, only the mechanism of his inspiration and guidance. Above all, he was a teacher, devoted to the molding of pathologists as well as clinicians; and, to those who were fortunate enough to work under his direction, he exemplified irreproachable standards of professional ability and intellectual honesty.

Born in 1874 in what was then Austrian Galicia, he was raised and educated in small towns. His family, relatively well-to-do merchants, was conspicuous in a Jewish community of dreary poverty, exposed to latent, and sometimes overt, anti-Semitism. According to a biography of E. M. Lilien, a playmate who later became a prominent graphic artist, the town was devoid of natural beauty and cultural advantages. At

JAKOB ERDHEIM, M.D.
1874-1937

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Thanks are due Drs. Albert Mueller-Deham and Leo Lowbeer for their assistance. Aware that any personal portrait is incomplete, the author welcomes any correspondence from Erdheim's pupils.

the age of 20, Erdheim began his medical studies in Vienna, graduating in April, 1900, when he joined the staff of the university pathological institute. Weichselbaum, then its chief, was interested in skeletal problems. Within a year, the new member published his first paper, devoted to branchial-cleft derivatives, with emphasis on the parathyroids. In 1908 Erdheim was named *Assistent*, and also clinical professor when he assumed additional duties at a children's hospital. Upon appointment as pathologist to the Vienna Municipal Hospital, in 1924, he achieved the status of full professor and also the right to conduct classes for academic credit. He died April 18, 1937. His last paper was posthumous (1938), coinciding with Hitler's march into Austria, when the last nails were driven into the coffin of Viennese medicine, born 150 years earlier.

What was Erdheim like? How did he work? These questions should be answered while his students can still recall the facts with reasonable clarity, and before time clouds the human outline in deceptive memory. Few facets of Erdheim, the man, were concealed from those of us who spent many months in his institute. It was 30 years ago that I first saw him; 5 years later I said goodbye to him for the second, and last, time.

In the large white-tiled autopsy room, Erdheim towered above the others. Despite his great height, his head was of normal size, his hair gray, fine, and closely clipped. The pale-brown eyes behind metal-rimmed spectacles could be at once demanding and disconcertingly expressionless. The face, in keeping with his disability, was an odd blend of adolescent boy and old woman, and the skin, covered by a delicate, barely perceptible fuzz, was soft and faintly brown.

His arms were disproportionately long, as were the broad fingers, which could be firm in grasp and delicate in touch. The wide pelvis easily supported the drawstrings of his white cotton trousers. Excessively long, his legs terminated in the largest pair of feet yet known to me. Over his clothing was a long yellow oiled-silk apron, which, thanks to his technique at autopsy, was rarely soiled by more than a few fine drops of blood. Thin white cotton gloves, such as were formerly worn by waiters, protected his rubber gloves.

He spoke a soft Austrian German in a pleasant tenor voice. Though he read English fluently, and apparently knew it well enough to arbitrate disputes between Americans over nuances in word meaning, he never spoke it, at least not in our hearing. This may have been due to that sensitiveness which made his public appearances so infrequent. I cannot recall his attending any professional meeting during my second stay with him. At that time, too, he refused an invitation to lecture in America; if he visited the United States, he said, hiding his shyness behind this subterfuge, it would be at his own expense.

His life centered in the hospital, and it was his home as well. In his quarters in the same building with the resident staff, the light shone late at night and before dawn on winter days. Beginning in secondary school, he had risen daily at 5 a. m., and this Spartan regimen he was prepared to recommend to his students. When I returned on my second visit, he arranged my schedule thus: one hour each for the three meals; seven hours of sleep (ashamed of my indolence, I had lopped off an hour). With paternal firmness, he settled my 14-hour working day.

By 7:30 a. m. Erdheim was already on duty, reviewing bacteriological material with a resident. As was common in the microbiological doldrums before the introduction of sulfonamides and antibiotics, this chore was somewhat cursory. Autopsies were begun at 8:00 a. m. and continued until they were completed, sometimes not before early afternoon. After a midday dinner, brought to his office, the Professor napped for an hour on a couch. Then he went over the surgical microscopic material with the residents, often telephoning the

surgeons, better to correlate anatomical and clinical findings. As a former student expressed it, he never operated in a clinical vacuum behind "the paraffin curtain." Afterward, he worked with persons engaged on special problems. Supper, too, was fetched from the hospital kitchens. This over, Erdheim either engaged in his own work or continued to supervise his students' efforts. Ofter it was after 10:00 p. m. before our weary mentor retired to his room, even then very likely to continue working.

Sunday was free of routine duties for him, although he occasionally looked into the necropsy room in the morning. On that day he might leave the hospital to visit members of his family, which included his brother, a surgeon. At no time did Erdheim boast the services of an associate pathologist, and once, with only a single resident on his staff, he alternated with his junior on Sunday necropsy duty for several months. Except for his holidays, this was the pattern of his existence.

The development of pathologic anatomy in Central Europe rested on certain social and statutory bases, primary among which was the legal right of administrators of governmental hospitals to order the examination of the bodies of any and all patients dying within their walls. Since, in Austria, there were few other hospitals, this meant virtually universal necropsy except for those dying at home. Constantly emphasizing the unique opportunities thus resulting to medicine, Erdheim was a vigorous critic of any procedure suggesting mutilation of the body, lest the laity take offense and seek to alter the law. As a case in point, a "buttonhole" in the skin of the neck would evoke a short, but blistering, reprimand: Maladroitness or carelessness was to blame, and either was reprehensible. The greatest skill was employed in removing the entire skeleton; in one case nothing remained except the face and finger and toe tips. The envelope of skin was then draped about a wooden scaffolding, and form and substance restored with sawdust packing. Embalming was a rare practice in Vienna.

On Erdheim's service, of the more than 2,000 necropsies performed annually, half came from the 1,000-bed municipal general hospital, of which the pathological institute was a division. The department also served adjacent institutions (old people's home and hospitals for chronic diseases), with a combined population of over 12,000 persons. Because of limitations of laboratory space and personnel, only a third of the 3,000 dying in those establishments were examined, and these cases were selected by the clinicians concerned with their care. I recall only a single day when the prosectors were without work, truly a red-letter occasion. More typical was another day: Erdheim, six necropsies; the chief resident, five, and another resident and I, each three.

Rarely was a clinical staff absent from necropsies on the bodies of its patients. Not only the interns and residents but the attending physicians and surgeons, and ordinarily also the heads of the services, were already on hand when the skin was incised. Erdheim, whom the other seniors greatly respected, usually asked questions. Thus, thanks to the clinical information and the ready gross anatomical diagnosis, each autopsy during its very performance became a clinicopathological conference. So it was that Vienna had achieved eminence as a center of medical education before two wars accomplished its destruction.

The head and bowel were routinely included in every postmortem examination; the assisting technician (*Diener*) or medical student removed the skullcap and opened the gastrointestinal tract after its removal from the body by the prosector. Lest artifacts be introduced into the gross appearance of organs, water was used sparingly. Instead, a well-wrung-out sponge collected blood and exudates and blotted cut surfaces. "Water," said Erdheim, "is a good servant, but nothing more." In an adjacent room, a band saw and frozen-

section apparatus afforded means for rapid study and diagnosis before the necropsy was completed. There were no dictating machines or stenographers; only several undergraduates were on hand to write down the protocols, consisting exclusively of diagnoses.

In common with the Viennese school of pathological anatomy, Erdheim stressed necropsy technique, careful observation, and gross anatomical diagnosis. Buttressed by his high intelligence and long experience, he maintained that microscopy is necessary only when something new appears: The microscopic picture once seen, diagnosis is made the next time with the unaided eye. "One trouble with American pathology," he commented, basing his observation on conversations with visitors from the United States, "is the reluctance to make diagnoses at the autopsy table. You ask the clinician to wait until the microscopic slides have been completed, forgetting that, while you have few autopsies, he has many patients. The clinician learns best while all details of the case are still with him, which means, at autopsy. A week or two later is too late."

An American student once challenged Erdheim's ability to make gross diagnoses in renal disease. Happily picking up the challenge, the Professor proposed that the visitor himself prepare sections from the next hundred bodies examined. (In preparation of sections, he always advised the pathologist himself to be competent in histological technique, so that he might criticize his technician's work from knowledge and experience, rather than from reading and hearsay.) When the task was done and diagnoses compared, Erdheim was wrong only once.

Whether of gross or microscopic findings, a description was to be a true image of the prosector's thinking. When the thinking included doubt or misgiving, it was obligatory to report this. "Otherwise," said the Professor, "the reader will assume you were positive, that you had no reservations. Of course, you can solve the dilemma by omitting all mention of that of which you are uncertain; at least, in that way the reader will not be misled." The intellectual honesty which prompted this insistence on clarity was modeled on that of his former chief, Weichselbaum. According to Erdheim, Weichselbaum's honesty was illustrated in one of his standard practices. Whenever microscopy uncovered the error of his own gross anatomical diagnosis, it was his custom to give both protocol and slides to a subordinate for completion of the report. This insured against any attempt of the ego to overlook or mitigate the contradictory evidence.

An autopsy over, the organs were carefully dried, spread out in a numbered metal pan, covered, and temporarily stored in the refrigerator, and the body removed. When all necropsies of the day had been completed, Erdheim seated himself on a tall stool before the marble-topped dissection table, where he examined each tray of specimens while the protocol was read aloud. He corrected and commented, interpolating, as an aid to memory, a few lines of the essential clinical and anatomical features. From the specimens he laid aside material for his undergraduate and postgraduate demonstrations, and for current and future research projects. At his suggestion, blocks were taken for infrequent microscopic study.

Each prosector was obliged to type his own reports, unless he could prevail upon a willing or an unresisting medical student to do it for him. Nowhere in the three-storied institute was a secretary employed, and, like his juniors, Erdheim did his own typing or victimized a subordinate for this purpose. In fact, there was only a single histology technician, who did the surgical pathology almost exclusively, interrupted by feuding with Erdheim. Inadequate assistance in the laboratory added up to an appalling waste of time and skill, chiefly his own, and was, in part, the price of a perfectionism which also rejected intellec-

tual waywardness and technical clumsiness. Undoubtedly, the perfectionism was aggravated by lack of those benign influences associated with a normal endocrine apparatus, and by the knowledge that men of lesser talents secured hospital and university posts denied him. This denial, in turn, could be ascribed to his psychic and physical constitution, and to recurring differences with hospital administrators. Finally, there was his religion; anti-Semitism had by no means died with the death of the Austro-Hungarian Empire in 1918. Once, in a rare display of that self-hate exhibited by minority groups, he asserted that Jews, in general, lacked the manual dexterity of non-Jews; this was probably the only false statement of major importance I ever heard him make.

From today's vantage point, various aspects of the institute's finances seem somewhat strange. The costs of all special studies, whether carried out by regular members or guests of the department, came not from the division's official budget but from a special fund which defrayed the costs of all supplies, as well as the pay of the *Diener*, who, in addition to his regular duties, made and stained the celloidin-embedded sections. The fund was supplied by payments from medical journals for articles from Erdheim's service. Such payment once was regular practice; *Virchows Archives*, for example, paid $5 to $10 for contributions, at the time a not inconsiderable sum, since it was equivalent to room rent for several months. The resources of the fund were incongruously housed in an old cigar box (Erdheim himself did not smoke) locked in a clothes closet in the Professor's office. From it came the pennies to reimburse a *Diener* for carfare or bank notes to settle accounts with large suppliers.

The initiation of a special project was preceded by long discussion with Erdheim. Believing with Claude Bernard that one case thoroughly investigated is worth many superficially pursued, the emphasis was on the quality of the study, not on the dimensions of the material. The field of research might be new; it might resurvey abnormalities in the light of newer methods of study, or it might include reexamination of diseases becoming less common and likely to disappear, a motivation responsible for reports on the osseous and articular features of syphilis. There was little animal experimentation, in part perhaps because Erdheim had come to believe the study of Man to be primarily Man, and the necropsy room provided ample proof to support him.

If a wide range of material was indicated, it was available. One American, supplied with pieces of skull from every age group, was able to compare microscopically the youthful expansion and the senile contraction of the cranium. I myself was set the task of reporting on cartilaginous and bony changes in the ankylosed knee joint; for this, two examples proved adequate. The two specimens were serially divided with the band saw; then each piece was radiographed, chiefly for orientation of the many subblocks from which the microscopic sections were made.

To save the investigator's time and labor, each structure encountered in the microscopic section had its own abbreviation; despite this, the survey of a single section might cover five closely typed pages, so thoroughly were the diggings mined. The student read the description aloud, adding and correcting as Erdheim reviewed the slide. Reading the last line with the satisfaction of a job well done, his joy was short-lived. Without raising his head from the microscope, Erdheim delivered the blow. "Now, let's write!" he said tersely, and, to the junior's astonishment, there followed another page or two of added description. To explain or apologize for the oversights was futile and self-defeating. Explanation only provoked a curt, marrow-chilling *"Wozu reden? Schade um die Zeit!"* ("Why waste time talking!") Of course, he was right; the omission had been made

good, and, hopefully, the next attempt would justify the teacher's patience.

At one "Let's write," the Professor described myriads of Russell bodies. Then I saw them for the first time, however often I might have looked at them. This was an invaluable lesson and illustrated the ego's tendency to ignore the existence of the unfamiliar, thereby "saving face" in the presence of a challenge. For this reason, much remains to be explored, even in more commonly encountered disturbances; hence, Erdheim's tireless reworking of seemingly routine conditions.

When the entire collection of slides had been reviewed, the microscopic elements and all page references to their descriptions were listed on a large accounting sheet. With its aid, gross, microscopic, and radiologic features fell into place. Only now was the literature explored, a precaution calculated to exclude prejudice at the outset of, or during the course of, the investigation. Then began the actual composition, which, for the foreigner who knew German only as a second or third language, was no easy task. Photomicrography was planned with Erdheim, and one of the residents served as camera man. After a reasonable time, the Professor called for the entire protocol, including the researcher's literary efforts, and a new period of gestation and waiting began. Then we knew why the lights burned early and late in his room. Before bed at night and before the 7:30 a. m. bacteriological review, Erdheim was writing our papers, even to the legends for the illustrations. For this best of all reasons, a student's publication could never be identified as that of a novice. Erdheim's name appeared only as director of the institute.

For this unofficial labor, he received not a penny from the student (who paid the hospital a few dollars a month for use of locker and laboratory coat), the hospital, or the university. In this, he was unlike his Central European colleagues, who usually charged good fees for their instruction. Of course, most of them were married and had families to support, while Erdheim's own worldly needs were extraordinarily modest. This he recognized, and he was, on the whole, unconcerned about the financial practices of others. Once, when I announced a projected trip to Berlin while he recuperated from an illness, he mentioned a well-known pathologist there with whom I might work. "Yes," he said, "you'll have to shove money down his gullet, but he's worth it!" Erdheim accepted as a student anyone who agreed to work on his terms. Each new associate meant an additional burden on him, but to this he never alluded.

This was Erdheim at work, but the picture would be incomplete without those features which fascinated, baffled, and bewildered his colleagues. In the absence of an audience, the mere performance of an autopsy had no meaning; to him, his time seemed wasted. For this reason, I sought, whenever possible, to serve as his assistant. During the 30-40 minutes of the necropsy, Erdheim at times talked without interruption, but customarily not about pathology. Only when something unusual was uncovered or I asked a question did he refer to the matter at hand. Once, as his fine scissors opened the common bile duct, he remarked: "The novice betrays himself in his dissection of passages. Either he stops at the obstruction, thus overlooking it, or else he plows through it and destroys it."

His monologues, a kind of obbligato to the necropsy, followed the lines of his nonprofessional interests—travel, history, and the natural sciences. One autopsy was devoted to an engrossing description of the life of a single species of tree ant (probably subsequent to an intensive reading of Fabre, a favorite author); another, to polar life. One dissection remains in memory only because of an amazed observation he made while examining the kidneys. He had spent the previous Sunday afternoon on a picnic (there is a photograph of him in Tyrolean costume, leather breeches, and all!), and

he was still astonished at the behavior of another guest. "Do you know what he did?" he demanded rhetorically, and, without waiting for an answer, "He picked up a tomato and ate it as you or I would an apple—without oil and vinegar!"

At another autopsy I spoke of an article on thyroid disease, done years before under his aegis; its author's name is now an eponym for a neurological disturbance. "Yes," Erdheim remarked, "when Schilder was staining sections, his laboratory coat rivaled Joseph's; but the results were beautiful." Other autopsies stimulated stories of his experiences in the First World War, when, for part of the conflict, he commanded a mobile laboratory. Knee-deep in Balkan snows, he performed necropsies while the guns thundered overhead. In a wartime publication, he explained the appearance of cholera among swimmers in the Adriatic Sea: The discharging wastes from transports and hospital ships depressed the salinity of the adjacent waters enough to permit survival of the vibrios. On another occasion scurvy broke out among the enlisted men; to save their own funds, the officers had been pilfering from the general mess. Erdheim announced a carefully planned antiscorbutic diet for the troops, adding drily: "I have never seen a single case of scurvy among officers." The pilfering stopped.

Like his work, his vacations were intensive. Before a visit to Rome, his nonmedical reading for two years was devoted exclusively to this city. Once there, he was on his way from sunrise to sunset. "A German tourist spoke to me in the Forum," he reported later, "and asked me where he was. Of course, I not only told him about the Forum, but even gave him the complete history of the very stone on which he stood." Knowing Erdheim, one could be sure the story was not apocryphal. Needless to say, he required a week's rest in a sanatorium before he could return to the institute.

Difficult though he was to live with, no one was more compassionate during another's illness. When a laboratory cleaning woman came down with appendicitis, the chief of surgery delayed starting his holiday to perform the operation; a junior surgeon would not do, the Professor told him. Let a student be indisposed, as I was, and Erdheim sent emissaries, advised medical care, and saw that the invalid was adequately nursed. But woe to the man who, on his recovery, assumed this to be the "basic Erdheim"; quickly, and with painful embarrassment, he learned his error.

He was not unconcerned with his own social problems, however, and occasionally sought to copy those who were more successful. As an instance, I once came across a long-unused croquet set in a laboratory closet. Erdheim had heard that Aschoff, whom he greatly respected, played tennis afternoons with staff members. In less vigorous emulation, he "persuaded" his juniors to join him in croquet, but he soon wearied of it. Nor was he lacking in the light touch. Once, in a mellow mood, he announced the criterion for skill at microscopy: when one can fall asleep at the microscope without betraying the fact—the head neither obviously falls back, nor falls forward to receive the firm, revealing imprint of the ocular on the offending eyelids.

Of the magnificent triangle of Erdheim's, the investigator and the mentor comprised two legs, the demonstrator of pathological anatomy, the third. Each Saturday morning, before a group of undergraduates, he held forth. Handling the specimens with loving care, he was a dedicated priest initiating the novice into the secrets of his calling. With his great frame bent over the table, he invoked the organs to take up their premortem activity before the onlookers' very eyes. Similar sessions were held periodically for foreign postgraduates, and as many as 80 doctors listened spellbound, despite no, or a minimal, knowledge of German; they, nonetheless,

carried away far more than the gist of the demonstration.

What was the secret of his great power as demonstrator and lecturer? He himself analyzed it well in "The Psychology of Postgraduate Teaching," his own title to the preface of his contribution ("Tuberculosis of the Calvarium") to the Libman "Anniversary Volumes." The postgraduate audience, he wrote, is composed of men varying in age, experience, knowledge, special interests, and intellectual receptivity, the last already diminished by the years since graduation. Further, he went on: "Each has tasted of the tree of bitter knowledge . . . [suffering] unavoidable but sensitizing mishaps and failures in practice." Then he described the psychic depression engendered by the failures, and the role of memory, sharpened by the emotional trauma, in helping to avoid similar future mistakes. Largely to escape the risks of this depression, the physician enrolls in postgraduate courses, where he bears no responsibilities and where the instructor presumably has already experienced the stings of error and the psychic penalties of misjudgment. Here, Erdheim believed, was the contradiction inherent in postgraduate teaching. The student has "the pleasure of learning without the accompanying psychic depression," the emotional requisite in the learning process. How to resolve the contradiction and compensate for the defects of learning-without-pain?

With this in mind, the lecturer approaches his audience of postgraduates, to whom, more than to undergraduates, he "must be a striking personality, for whom teaching has become a passion. It is personality which works upon and impresses one's fellows. Because his auditors no longer enjoy pristine memory, the instructor . . . must so impregnate the dry, purely matter-of-fact with his personality that the two remain intimately associated. It is by using this haptophore group, so to speak, that he secures retention in the hearer's mind."

Between my two visits, Erdheim contracted typhoid during an epidemic initiated by the faulty water supply of a Viennese dairy, whose owner's two sons, ironically, perished. When I returned in 1932, I found him in a suburban sanatorium, this time with a "top-secret" illness. The residents hinted at peptic ulcer. Only years later, in America, I learned it was tuberculous pleuritis, a condition which incapacitated several members of his staff over the years. It was during this illness that one day it was my lot to bring Erdheim his mail, slides for review and opinion, as well as the rare biopsy specimen which comprised his meager private practice. After he had cut a small object into blocks, he asked me whether I knew the identity of the patient. I shook my head. "It comes from the mouth of Professor Freud," he said. "If that man doesn't stop smoking, he's going to die of cancer." He did, but he outlived his pathologist by two years.

Erdheim died in his sleep while his medical attendant was absent from Vienna. Accordingly, there was no one legally qualified to sign the death certificate, and the autopsy was performed at the Medicolegal Institute. The subject of the necropsy would have been chagrined to know that postmortem changes were already advanced; death probably came hours before, in a warm room. His temper would not have been improved by the knowledge that the skull was not opened. The heart (none of the organs was weighed) was large; coronary ostia were not narrowed, but the arterial lumens were of hair's width, thanks to calcific sclerosis. A fresh thrombus occluded the descending branch of the left coronary artery, accompanied by fresh infarction; myocardial scarring was also recognized. Nephrosclerosis was present.

The penis was small (2×1.5 cm.), as was the glans. The testes, located in the inguinal canals close to their exits, were infantile (bean-sized) and composed of "pale-gray, gelatinous" tissues. It was reported that the prostate was strikingly

small, and each spermatic cord was infiltrated with fat. While the right breast was grossly made up of fat, the left also included gray tissues, apparently glandular.

Anginal symptoms, present on effort for several years, had responded well to the usual treatment. The patient disagreed with the diagnosis of coronary arterial disease, insisting that the pain was produced by fibrosis, secondary to the former pleuritis, which involved the vagus nerve. It is obvious that this fanciful explanation was designed to avoid enforced changes in his normal existence; at the time of his death, Erdheim was working 16 hours a day.

The single physical memento of Erdheim consists of a fine collection of dried bone specimens, which I labeled for him 30 years ago. It was originally bequeathed to a brilliant pupil (Dr. Ernst Freund), who died in America before he could receive it. By this time, the Germans had taken over Austria, and the collection was included in the personal baggage of another student, who brought it to Dr. Henry L. Jaffe, of New York, to whom it had been willed by the original legatee.

Like Freud, Erdheim and his great Viennese colleagues exercised an inestimable, world-wide influence on medicine. With their passing, and with the coming of the Nazi barbarians, Vienna disappeared as a great medical center. Yet Erdheim's influence remains to me, as it does to all his students, the prime mover in my professional life. Deprived of many of the normal pleasures, and without obvious neurotic conflict, he dedicated himself to science. His deprivation was Medicine's gain.

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Granulomatous Myocarditis and Myositis Associated with Thymoma

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The present case of granulomatous giant-cell myocarditis and myositis, associated with a thymoma, is reported because of its rarity, only two other such cases [8,14] having been encountered in a review of the literature.

Report of a Case

Clinical Data

History.—A 75-year-old white woman was admitted with a history of intermittent nausea, vomiting, and epigastric pain for two years, and dyspnea on exertion and some orthopnea for one year. In the month prior to admission she had been confined to bed because of severe dyspnea and had lost 4 or 5 lb. in weight. She had been given digitalis four months prior to admission, without apparent benefit.

Physical Examination.—Blood pressure 100/80; temperature 100.2 F rectally; pulse rate 110 and respiratory rate 22, per minute. Crepitant rales were heard over the base of the left lung. The area of cardiac dullness was enlarged to the left; no murmurs were heard, and the heart sounds were of good quality. The abdomen was soft, and the liver was palpable 2 fingerbreadths below the right costal margin.

Laboratory Data.—Examinations of blood cells and urine were within normal limits. Sedimentation rate, 102 mm. in 60 minutes; hemoglobin, 11 gm. per 100 ml. of blood; hematocrit reading, 31 vol. %; mean corpuscular volume, 86 cu. μ; mean corpuscular hemoglobin, 28$\mu\mu$; mean corpuscular hemoglobin concentration, 36%; nonprotein nitrogen, 40 mg. %; circulating eosinophils, none. Electrocardiographic impression: left bundle-branch block; T-wave changes indicating severe myocardial damage. The changes seen in Leads II, III, and aVF suggested the possibility of a posterior myocardial infarction.

Course in Hospital.—On the evening following admission, the patient went into shock and died.

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From the Registry of Unusual Cardiac Lesions (S. E. Gould, M.D., Director), Department of Pathology, Wayne State University College of Medicine.

Dr. Langston is Pathologist, Detroit Memorial Hospital; Dr. Wagman was Resident in Pathology, Wayne County General Hospital, Eloise, Mich., and is now Pathologist, St. Joseph's General Hospital, Brantford Ont., Canada; Dr. Dickenman was Resident in Pathology at Receiving Hospital of the City of Detroit, and is now Associate Pathologist, Detroit Memorial Hospital.

Gross Autopsy Findings

The *thyroid gland* weighed 30 gm. A firm gray nodule, measuring 1.5 cm. in diameter, was present at the lower pole of the left lobe. The right *lung* weighed 385 gm., and the left, 370 gm. The left pleural cavity was obliterated by fibrous adhesions, and the right pleural cavity contained 100 ml. of serous fluid. An encapsulated mass in the region of the *thymus* measured 8×6×4 cm., composed of soft gray tissue, and contained ragged cystic spaces, measuring up to 1.5 cm. in diameter. The *heart* had a globular shape, and the left ventricle was dilated. The cardiac formula was 90-185/465 (Stofer and Hiratzka [13]). The left ventricular wall measured 1.5 cm. in thickness. Large pale-gray areas were present in the lateral wall of the left ventricle. The valves were not remarkable, and the epicardium was smooth and glistening. The anterior portion of the left *deltoid muscle* was normal in configuration but firm, pale gray, and fibrous. There was Grade II to Grade III coronary atherosclerosis. The *spleen* weighed 270 gm. and presented a smooth capsule and a soft pale red-gray parenchyma, without prominence of follicles or trabeculae. The *liver* weighed 2,500 gm. and the parenchyma was finely mottled and red-brown. The *ovaries* measured 3×2×2 cm. and presented multiple cysts containing amber fluid, the largest cyst measuring 2 cm. in diameter. In the right *putamen* a few slit-like spaces, the largest 3 to 4 mm. in length, were present. There was generalized, Grade II to Grade III cerebral atherosclerosis. No other significant gross findings were present.

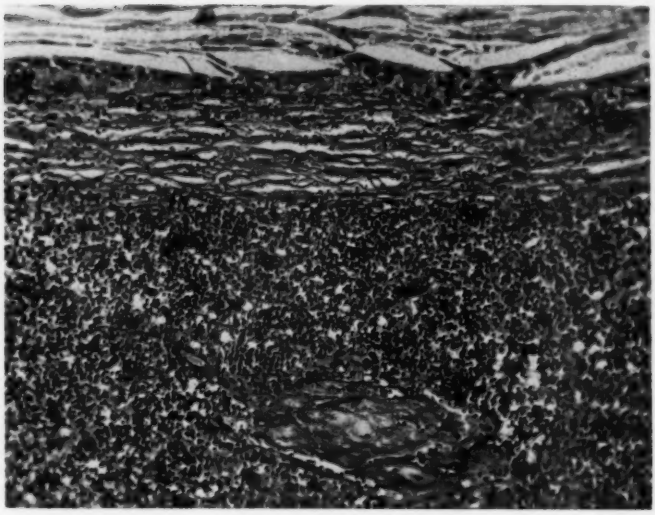

Fig. 1.—Section of thymus showing diffuse sheets of lymphocytes interspersed with occasional reticular cells. Note part of wall of cholesterol cyst in upper portion of field. Hematoxylin and eosin; reduced to 80% of mag. × 120.

Microscopic Autopsy Findings

The *thyroid gland* presented a nodular appearance. Groups of thyroid follicles of various sizes were separated from one another by septa of fibrous tissue, which were heavily infiltrated with lymphocytes. In some sections heavily infiltrated with lymphocytes the parenchyma consisted of cords and acini of Hürthle cells. No lymphoid follicles were present. A large encapsulated nodule was composed for the most part of relatively small follicles, containing unequal amounts of colloid.

The *thymic* mass consisted of diffuse sheets of lymphocytes with occasional interspersed reticular cells (Fig. 1). Many septa of dense collagenous tissue were present, with areas of calcification, fat-laden macrophages, and cholesterol clefts. The fibrous capsule was intact and not invaded by neoplasm.

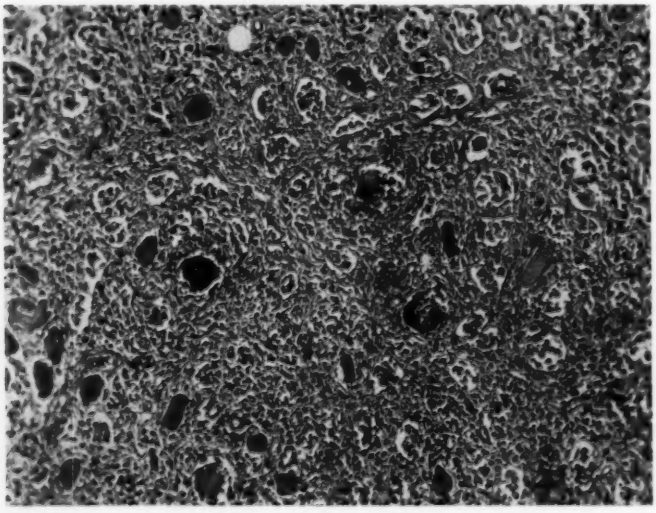

Fig. 2.—Section from left deltoid muscle showing remnants of muscle fibers. The great majority of fibers are represented by rings of sarcolemma, each enclosing a variety of inflammatory cells and some, a multinucleated giant cell. Hematoxylin and eosin; reduced to 80% of mag. × 160.

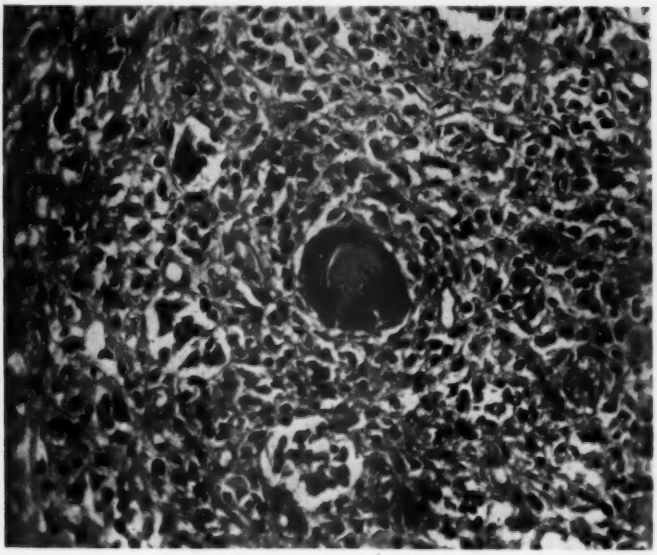

Fig. 3.—Higher magnification of Figure 2, showing granulomatous nature of the lesion. The large multinucleated giant cell encloses a fragment of degenerated muscle fiber. Hematoxylin and eosin; reduced to 92% of mag. × 340.

The left *deltoid muscle* presented only a few scattered normal muscle fibers. The great majority of fibers were represented by tubular rings or bags of sarcolemma, each ring or bag surrounding a variety of cells, chiefly histiocytes, lymphocytes, and many giant cells (Figs. 2 and 3). The giant cells were oval and occasionally spindle-shaped, and each contained from 3 to 25 nuclei. The nuclei were situated both centrally and peripherally throughout the cell. Occasional giant cells were present only within the sarcolemmal bags and occasionally were intimately attached to the sarcolemma. The endomysial sheaths were converted into greatly thickened strands of collagen and were bordered by proliferating fibroblasts,

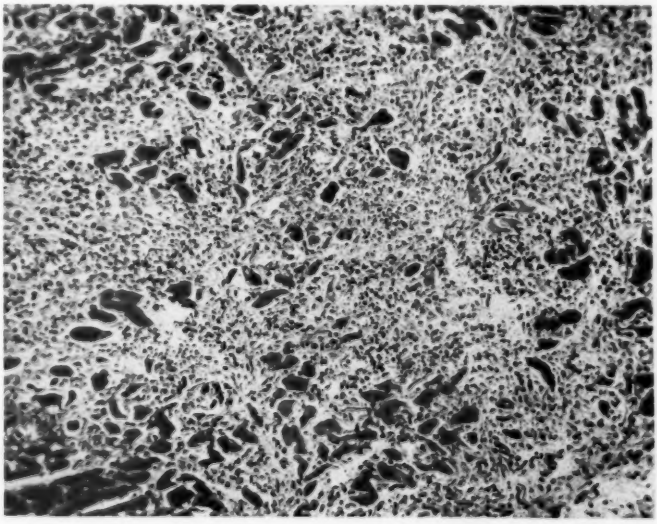

Fig. 4.—Section from left ventricle showing replacement of myocardial fibers by granulomatous tissue. Note numerous bizarre multinucleated giant cells. Hematoxylin and eosin; reduced to 86% of mag. × 85.

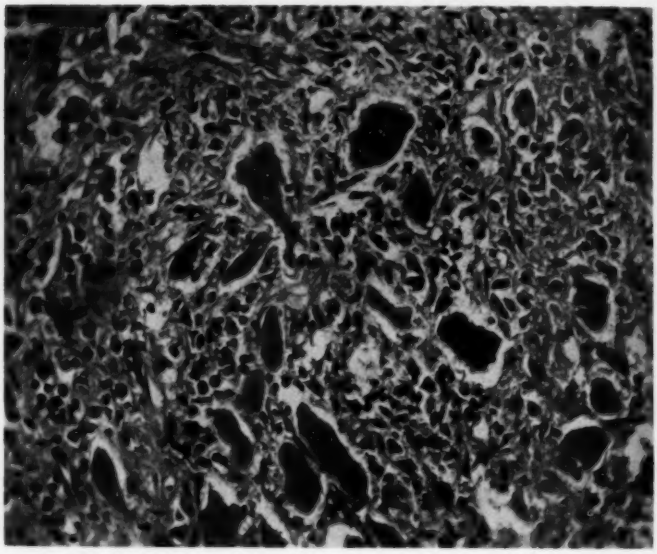

Fig. 5. — High-power magnification of a granulomatous area in the myocardium. Note the pleomorphic giant cells and heavy inflammatory infiltrate. Hematoxylin and eosin; reduced to 92% of mag. × 340.

many histiocytes, plasma cells, and lymphocytes.

Sections of the *left ventricle* showed replacement of myocardial fibers by focal, often confluent, areas of granulomatous tissue (Figs. 4 and 5), which did not involve the epicardium but extended up to the endocardial surface. These areas consisted of numerous bizarre multinucleated giant cells, plasma cells, histiocytes, Anitschkow myocytes, lymphocytes, and fragments of degenerated myocardial fibers. No eosinophils were present, but tissue mast cells were moderately increased in number. There was no suggestion of tubercle formation or caseous necrosis and no perivascular pattern. The giant cells were of various sizes and shapes, with oval and spindle-shaped forms predominating. Many narrow, elongated, tapering forms, measuring up to 300μ in length, were present. The cytoplasm of these cells stained faintly basophilic, and almost all contained lipochrome granules. Many showed large, faintly eosinophilic, round or oval, granular areas within their cytoplasm. The nuclei varied in number from 3 to 50 and were pale and vesicular, with prominent nucleoli. Many giant cells showed peripheral rings of nuclei resembling Langhans giant cells, while others showed a central aggregation of nuclei resembling foreign-body or muscle giant cells. Giant cells were often seen in close relation to the frayed ends of myocardial fibers, and the cytoplasm of occasional giant cells appeared to envelope small fragments of degenerated muscle fibers. However, an intervening clear space was always present between the fragments of degenerated muscle and the cytoplasm of the giant cell. Occasionally a small focus of polymorphonuclear leukocytes was associated with degeneration and fragmentation of muscle fibers. In these foci no giant cells were found.

Special stains of the muscle lesions could be performed only on the cardiac lesion, since paraffin blocks or formalin-fixed skeletal muscle was not submitted. Stains for acid-fast bacilli, bacteria, fungi, and spirochetes were negative. Iron-hematoxylin and phosphotungstic-acid-hematoxylin stains did not reveal striations within the cytoplasm of giant cells.

Other autopsy findings included a small cystic infarct of the right putamen, pulmonary congestion and edema, congestion of

the liver, chronic splenic hyperplasia, and bilateral ovarian follicular cysts.

Comment

Saphir,[10] who reviewed the subject of myocarditis in 1941, made a distinction between the so-called isolated myocarditis (Fiedler's myocarditis) and myocarditis with granulomatous lesions. He and Cohen [11] also indicated that the viral form of myocarditis is associated with endocardial and epicardial inflammation, while Fiedler's myocarditis is not. Lichtenberger [7] (1957), on the other hand, grouped all cases of myocarditis of unknown etiology together as isolated myocarditis. In his nine reported cases, he included an instance of chronic diffuse granulomatous myocarditis with giant-cell formation in association with an endocardial lesion (Case 8).

Goldberg [6] (1955) reported a case of giant-cell myocarditis in a 2½-month-old boy. The heart exhibited two different lesions. One lesion consisted of areas of calcification surrounded by necrotic myocardial fibers; the other, formation of granulation tissue containing multinucleated giant cells, in some of which nuclear inclusion bodies were demonstrable. He believed that the giant cells represented an attempt at regeneration of the myofibrils.

A survey of the literature revealed only two cases of granulomatous giant-cell myocarditis and myositis in association with a thymoma. Waller and associates [14] found this unusual triad of findings in a 64-year-old white woman who presented herself clinically with a postmenopausal muscular dystrophy. The pathologic lesions in the cardiac and skeletal muscle appear to be identical with those in the present report. These authors emphasized the clinical and pathologic resemblance of Fiedler's myocarditis to the heart disease of muscular dystrophy and stated that there were no reports in the literature describing the skeletal muscle in patients with Fiedler's myocarditis. Mendelow and Genkins [8] reported a similar triad of findings in a 37-year-old white woman who clinically had an anterior mediastinal mass and symptoms of myasthenia gravis.

Granulomatous myositis has been described in both progressive muscular dystrophy [1] and myasthenia gravis.[5,8,9] Although similar changes in the cardiac musculature in both of these conditions are not uncommon, multinucleated giant cells have been described in the cardiac lesions only in the cases mentioned above.

The relationship of thymoma and thymic hyperplasia to myasthenia gravis is well known.[8] In progressive muscular dystrophy, subinvolution of the thymus has been frequently noted.[2] Although the case reported in this paper presented no clinical evidence of a disorder of skeletal muscle, it is possible that this may have been overlooked, owing to the short period of hospitalization and the predominant and rapidly progressive cardiac symptoms. Indeed, Waller and associates [14] mention skeletal muscle disease as a possible cause for the absence of detected association of Fiedler's myocarditis with skeletal muscle disorders. Although at autopsy only one skeletal muscle was described as being involved in this case, involvement of others may easily have been overlooked.

The skeletal muscular lesion in the present case corresponds closely to Bevans' description,[2] in two cases of progressive muscular dystrophy, of "peculiar channel-like fibrous structures" surrounding fragments of degenerate muscle. These structures appear to correspond to the tubular rings described in the present case.

Some authors have speculated on the nature of the multinucleated giant cells occasionally found in Fiedler's myocarditis. Tesluk [13] found striations within giant cells in his case and believes that these cells are of myogenic origin. However, as Dilling [4] pointed out, "the presence in the giant cells of inclusions having the same density and staining property as striated material could as well be regarded as an indication of phagocytic activity as of metamorphosis."

She believes that transition forms between muscle fibers and giant cells must be demonstrated in order to prove a myogenic origin.

In our material, no striations could be demonstrated within giant cells in the myocardial lesions. The skeletal lesion could not be studied with special stains, owing to the absence of submitted tissue.

Giant cells of muscle origin are frequently observed in skeletal muscle. They are regarded as a manifestation of attempted regeneration and represent proliferation of sarcolemmal nuclei.[1] They often form club-shaped sprouts attempting to bridge gaps in sarcoplasm. It is possible that the giant cells in the skeletal lesion in this case are of this nature. However, the lesion is so destructive in nature, without any apparent evidence of regeneration, that it is also possible that the giant cells are of phagocytic nature. Indeed, the cytoplasm of many cells, instead of being regenerating sarcoplasm, appears to enclose degenerated muscle fragments.

It is thought that, in contrast to skeletal muscle, cardiac muscle does not regenerate significantly. Consequently, it would appear more likely that the giant cells in the cardiac lesions are of a foreign-body, phagocytic type. If this is true, it can only be postulated that in the giant-cell variety of isolated myocarditis, myocardial fibers undergo an unusual type of death, requiring multinucleated phagocytes to clear the debris.

We referred the microscopic sections to Dr. Otto Saphir, of Chicago. He was of the opinion that the myositis and myocarditis were incidental findings, in no way connected with the thymoma; that the patient had the so-called granulomatous variety of myocarditis, and that perhaps the same agent that caused the myocarditis had caused the myositis. He also mentioned a case of his own of thymoma which was associated with giant-cell myositis but no myocarditis.

In conclusion, it is recommended that in all autopsied cases of isolated myocarditis the thymus and skeletal muscle should be especially studied. Similarly, a detailed study of the heart is indicated in all skeletal muscular disorders.

Summary

A case of granulomatous myositis and myocarditis associated with a thymoma is reported. Only two similar cases were found in the literature. The nature of the giant cells in the myocardium and the relation of the thymus to lesions of both skeletal and cardiac muscle are discussed.

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Arrest of Mitotic Division in Ehrlich Tumor by Extracts of Human Breast Tissue

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Recently we observed that when Ehrlich ascites tumor cells were implanted subcutaneously, they grew much more rapidly in CAF_1 hybrid than in Strong A mice, although no such difference was detected in growth rate of the tumor when the cells were implanted in muscle or in the peritoneal cavity of these two strains of mice.[1] Investigation of this observation showed that the subcutaneous tissues of Strong A mice had responded to implantation of the Ehrlich tumor by forming a fibroblastic pseudocapsule and by producing a polysaccharide-like substance that inhibited mitosis and temporarily suppressed growth of the tumor. The subcutaneous tissues of CAF_1 hybrid mice, on the other hand, showed neither of these reactions.

Because the microscopic appearance of the fibroplasia about the tumors implanted in Strong A mice resembled closely that of the fibroplasia of scirrhous adenocarcinoma of the human breast, it seemed worth while to investigate whether or not the stroma of human carcinomas also contains a substance that inhibits mitosis. Accordingly, extracts were made of both scirrhous and medullary carcinomas, as well as of several benign lesions of the breast, and were tested for their ability to arrest mitotic division of Ehrlich ascites tumor cells. Extracts of the scirrhous carcinomas showed such mitosis-inhibiting properties, but extracts of the other breast lesions did not.

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Material and Methods

Extracts were made from three scirrhous adencarcinomas, one medullary adenocarcinoma, one fibroadenoma, normal breast tissue, and tissue from one case of fibrocystic disease. These tissues were obtained from women at the time of breast surgery. In addition, tissue was obtained from one man with gynecomastia.

The fresh tissues were either extracted immediately or stored in the deep-freeze and extracted later. The method of extraction was a modification of the one we had used previously to extract mouse tissues.[3] A brei was prepared by grinding the fresh tissue with an equal volume of distilled water in a Waring Blendor for 20 minutes. Protein was precipitated from the brei with chloroform and isoamyl alcohol according to the method of Sevag.[2] The filtrate was defatted with an equal volume of chloroform and the aqueous portion extracted twice more with ether. The pH of the resulting aqueous solution was adjusted to between 7.4 and 8. The solution was dialyzed against distilled water for 24 hours at 4 C and then lyophilized to a grayish or yellowish-white powder.

The Ehrlich ascites tumor used in the experiments was propagated from a line obtained in 1953 from Dr. Robert Schrek, of Veterans Administration Hospital, Hines, Ill., and maintained since then by weekly intraperitoneal transfers of 0.2 ml. aliquots of the ascitic suspension into Strong A mice of both sexes when they were 3 to 6 months old. The Strong A mice were from stock animals originally supplied by Dr. Guy P. Youmans, Department of Microbiology, Northwestern University Medical School. They were caged in air-conditioned rooms and fed as much Rockland Mouse Pellets and water as they desired.

The tissue extracts from the various breast lesions were screened for their ability to reduce cytoplasmic viscosity by an vitro test described previously.[3,4] Briefly, 3- to 6-month-old Strong A mice were inoculated intraperitoneally with Ehrlich tumor. After a 7- to 14-day growth, the ascitic fluid was aspirated, and 0.5 ml. was placed in each of three plastic tubes. Nothing was added to the first tube; the tissue extract was added to the

second tube, and isotonic saline was added to the third. The number of polar cells * in 500 cells was counted in the first tube. The samples in the second and third tubes were centrifuged at 9,900×G for 30 minutes at 0 to 4 C, using the high-speed head of a refrigerated International centrifuge (Model PR-2). The number of polar cells was counted in both tubes after centrifugation. The relative viscosity value (VV) was derived by comparing the percentage of polar cells in the aliquot of untreated and uncentrifuged cells with the percentage of polar cells in the aliquot of treated and centrifuged sample.

$$VV = \frac{\%\text{ polar cells, untreated and uncentrifuged}}{\%\text{ polar cells, treated and centrifuged}}$$

In the in vivo experiments Strong A mice, 3-6 months old, were inoculated intraperitoneally with Ehrlich tumor, and after 7 to 14 days a 0.1-0.2 ml. aliquot of ascitic fluid was removed and the initial viscosity value (VV) of tumor cells determined, as outlined in the in vitro test. The number of mitoses per 1,000 cells was counted (mitotic index) on the same sample, using a procedure previously described.[5] The mice were injected intraperitoneally with the tissue extracts, and, at known intervals, samples of the ascitic fluid were withdrawn and the mitotic indices and viscosity values of the tumor cells measured. These measurements were compared with those obtained immediately before treating the mice with the extracts.

The average dose given was 30μg. of the dialyzed and lyophilized extract. This dose was determined by testing the viability of the cells in vitro following exposure to varying concentrations of the extracts according to the Schrek eosin method.[6]

Simple chemical tests for carbohydrates have been performed on the crude extracts. One- to two-milligram portions of the lyophilized extracts were dissolved in water and hydrolyzed with N HCl or N H_2SO_4 at 23C for 24 hours. Fehling's, Seliwanoff's, and Molisch tests were performed on the hydrolyzed extracts.[7]

* A polar cell is one in which all the lipid granules in that cell are located at one pole in an area no greater than one-third the total area of the cell.

Results

I. Effect of Extracts on Protoplasmic Viscosity of Ehrlich Tumor in Vitro.—Table 1 gives the results obtained in the in vitro experiments. It shows that the mean viscosity value of Ehrlich tumor cells was reduced significantly when they were exposed for 15-30 minutes to extracts prepared from breasts with scirrhous adenocarcinomas. The viscosity values of the treated tumor cells ranged from 0.61 to 0.69, as compared with the value for the untreated controls, which remained unchanged at 0.95. The other extracts did not alter the viscosity of the tumor. This was true even for the extracts of a medullary adenocarcinoma and a fibrocystic breast, where the dose was increased from the usual 30μg. to 50μg.

II. Effect of Extracts on Protoplasmic Viscosity and Mitotic Index of Ehrlich Tumor in Vivo.—A. Extracts from scirrhous Adenocarcinomas: The results obtained using the extracts from three different scirrhous carcinomas are presented in Table 2. The mean viscosity value at 0 time was 0.97. Fifteen minutes later the viscosity value dropped to 0.64, after which it gradually increased and returned to normal after five hours.

TABLE 1.—*Effect of Various Human Breast Tissue Extracts on Protoplasmic Viscosity of Ehrlich Ascites Tumor Cells in Vitro* *

Source of Extract	Extract Labeled	No. of Animals	Amount of Extract Used per Test, μg.	Mean VV	Standard Error
Scirrhous carcinoma	A	7	30	0.69	0.02
Scirrhous carcinoma	F	12	30	0.63	<0.02
Scirrhous carcinoma	H	11	30	0.61	0.03
Medullary carcinoma	D	9	30-50	0.92	0.02
Fibroadenoma	G	10	30	0.93	<0.01
Fibrocystic disease	E	10	30	0.94	0.03
Gynecomastia	B	5	30	0.95	0.02
Normal breast tissue	C	9	30	0.96	0.02
Untreated control	—	56	--	0.95	<0.01

* Duration of exposure: 15-30 minutes before centrifugation.

TABLE 2.—*Effect of Tissue Extracts from Scirrhous Adenocarcinomas of Breast on Viscosity Values of Ehrlich Ascites Tumor Cells in Strong A Mice in Vivo*

Extract Labeled	Animal No.	Hours								
		0	¼	1	2	3	4	5	18	24
A	1	1.04	0.71	0.76	0.75	0.90	0.96	1.03	0.87	
A	2	0.95	0.71			0.82		1.03		
A	3	1.02	0.73					1.04		
A	4	0.94	0.69	1.02	1.05					
A	5	1.07	0.63	0.52		0.85				
A	6	0.97	0.66	0.61		0.93				
A	7	0.91	0.70	0.69		0.94				
F	8	0.92	0.56	0.73		0.83		0.92		0.97
H	9	0.96	0.46	0.73						0.91
H	10	1.02	0.63	0.90						0.92
H	11	0.97	0.54	1.04						0.96
H	12	0.93	0.66	0.75						0.93
Mean		0.97	0.64	0.77		0.86		0.97		0.94
S. E.		<0.01	0.02	0.06		0.03		0.03		<0.01

Table 4 shows the rise of the mitotic index of tumor cells following treatment with extracts of scirrhous adenocarcinomas. At 0 time the mean mitotic index was 1.8. At the end of 6 to 8 hours the mitotic index was 3.5, and a peak of 4.8 was reached after 18 hours. After 30 hours it declined to slightly above normal. Examination of the treated cells with the phase-contrast microscope disclosed that many of the dividing cells were in metaphase. The viscosity values and mitotic indices at various time intervals following treatment with extracts from scirrhous carcinomas are depicted graphically in Figure 1. This Figure shows that the increase in mitotic index occurred six to eight hours after the drop in protoplasmic viscosity.

B. Extracts from Other Breast Lesions: Addition of these extracts had no effect on the viscosity values, as shown in Table 3, or on the mitotic index (Table 5). These results are shown graphically in Figure 2.

In all in vivo experiments the dose of tissue extract was 30μg. Higher doses were not used, since we had previously determined that they reduced the viability of the tumor cells by as much as 40%-50% when tested by the eosin method. In no case, however, did a dose of 30μg. affect viability of the ascites cells.

III. Chemical Properties of Tissue Extracts.—Preliminary studies of the chemical characteristics of the hydrolyzed extracts obtained from scirrhous carcinomas of the human breast showed they reduced Fehling's solution and gave a positive Molisch reaction, but were negative for ketone configuration by Seliwanoff's test. Unhydrolyzed extracts gave negative results with tests for

TABLE 3.—*Effect of Tissue Extracts from Nonscirrhous Breast Lesions on Viscosity Values of Ehrlich Ascites Tumor Cells in Strong A Mice in Vivo*

Extract Labeled	Animal No.	Hours					
		0	¼	1	3	6-8	24
B	1	0.91	0.97	0.97	0.93	0.97	0.91
B	2	0.92	1.03	0.94	0.97	0.94	0.97
C	3	0.93	0.97				1.05
C	4	0.94	0.84				0.88
D	5	0.93	0.90				1.03
D	6	0.91	0.96				0.88
E	7	0.97	1.02				0.90
E	8	1.06	0.94				0.89
Mean		0.95	0.95				0.94
S. E.		0.04	0.02				0.02

TABLE 4.—*Effect of Tissue Extracts from Scirrhous Carcinomas of Breast on Mitotic Index of Ehrlich Ascites Tumor Cells in Strong A Mice in Vivo*

Extract Labeled	Animal No.	Hours 0	¼	1	3	5	6-8	9	12	15	18	24-30	30-On
A	1	2.2			1.6	1.4	4.0	5.1				5.5	2.9
A	2	1.8			1.0	0.3	4.8				4.1	3.2	2.5
A	3	1.4			1.0	0.9	1.5					4.7	
A	4	1.9	2.4	2.6		2.8	5.4	3.8				3.0	2.8
A	5	1.3					4.2						
A	6	1.7		3.5		2.5	4.7	5.5	7.3	5.5		4.6	2.9
A	7	1.7				1.0	1.2	2.4	1.5			2.0	
F	8	1.7							5.2	4.2	4.2		2.2
F	9	0.8							2.3	3.0	5.0		2.6
F	10	3.6							4.3	4.6	5.9		1.4
F	11	1.9	2.1		1.2	2.1	2.1	2.2	3.4			3.0	
H	12	1.6					3.8					2.5	
H	13	2.1					3.0					1.7	
H	14	1.8					3.1					2.0	
H	15	2.4					4.2					1 8	
Mean		1.8			1.2	1.6	3.5	3.8	4.0	4.3	4.8	3.0	2.4
S. E.		0.04			0.02	0.1	0.4	0.3	0.3	0.1	0.5	0.3	0.2

simple sugars, a result which was not surprising, since the crude extracts had undergone dialysis during preparation.

Comment

Cell physiologists generally agree that the successful completion of mitotic division is, in part, dependent upon the development and activity of normal spindle fibers. In the collodial theory of cell division, the mitotic spindle is assumed to be a solid structure arising from the fluid protoplasm as a result of gelation.[8,9] Theoretically, if gelation could be prevented, no spindle formation could occur. Heilbrunn and collaborators[10-12] have investigated inhibition of cell division by decreasing the protoplasmic viscosity in nonmammalian cells. Previously, we pre-

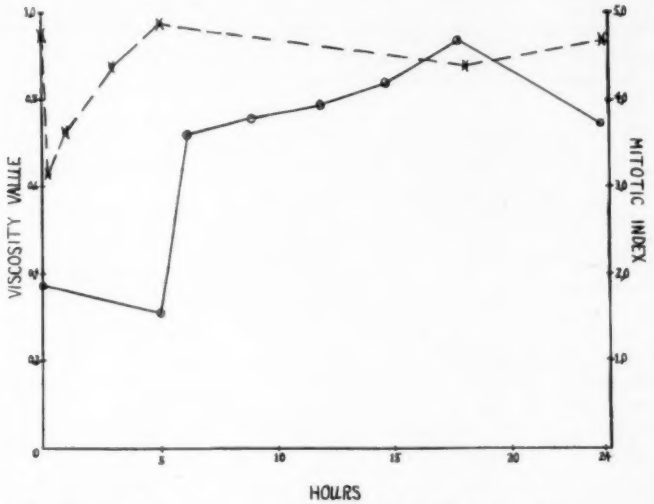

Fig. 1.—This figure depicts the response obtained when Ehrlich ascites tumor was treated with extracts from human breasts the site of scirrhous adenocarcinoma. The viscosity values are shown by the broken line, and the mitotic index, by the solid line. The treatment was begun at 0 time.

TABLE 5.—*Effect of Tissue Extracts from Nonscirrhous Breast Lesions on Mitotic Index of Ehrlich Ascites Tumor Cells in Strong A Mice in Vivo*

Extract Labeled	Animal No.	Hours			
		0	3	7	24
B	1	1.3	1.1	1.0	1.2
B	2	0.8	1.3		1.1
C	3	1.1		1.3	0.9
C	4	1.0		1.4	1.5
D	5	1.1		1.3	0.9
D	6	1.8		1.3	1.2
E	7	2.1		1.1	0.9
E	8	1.0		1.3	1.5
Mean		1.3		1.2	1.1
S. E.		0.19		0.05	0.09

sented evidence of a similar mechanism in mammalian cells, suggesting a relationship between mitotic inhibition and alterations in cytoplasmic viscosity of Ehrlich ascites tumor cells.[1,3,4] The data showed that complex polysaccharides capable of reducing viscosity could inhibit cell division. Heilbrunn and his co-workers[10,11] noted similar effects of other polysaccharides from vertebrate sources on the cleavage of sea urchin eggs. These findings led us to investigate the possible presence in human tissue of polysaccharides capable of inhibiting mitotic division of Ehrlich tumor cells.

Our studies, under the conditions of our experiments, showed that human breast carcinomas associated with a marked fibroblastic response contained a polysaccharide complex which temporarily reduced cytoplasmic viscosity and inhibited mitosis of Ehrlich tumor cells. The adenocarcinoma without marked fibroplasia, on the other hand, did not contain such a material. Moreover, the extracts of benign lesions and normal breast tissue did not have any effect on viscosity and mitosis. The results obtained from human tissue extracts are similar to those from mouse tissue extracts,[1] Serratia marcescens polysaccharide, and colchicine[3,4] except for the longer latent period observed prior to mitotic arrest. Since the extract of a fibroadenoma did not produce any mitosis-inhibiting material, the host response of marked fibroplasia appears to be specific for certain malignant neoplasms of the human breast. Another possible explanation is that there may be a biochemical difference in the activity of the fibrous tissue in fibroadenomas, as compared with the fibroblastic carcinomas. As in the case of the mouse experiments,[1] we can only postulate that the viscosity-reducing polysaccharide complex is produced by the proliferating fibrous tissue.

It will be noted in Figure 1 that the reduction in cytoplasmic viscosity precedes mitotic arrest of tumor cells by an interval

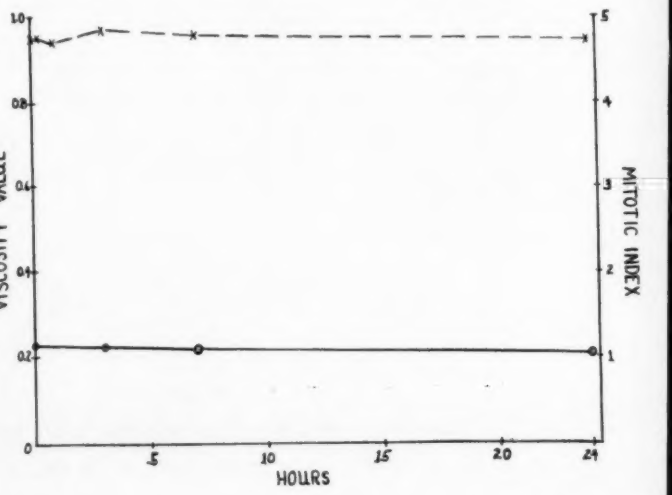

Fig. 2.—The viscosity values are shown by the broken line, and the mitotic index, by the solid line. This graph depicts the response obtained after treatment with extracts of normal breast, benign breast lesions, and a nonscirrhous adenocarcinoma of the breast. No change is detected.

of five hours. The increasing values of mitotic index obtained correspond roughly to the theoretical multiplication rate of the Ehrlich tumor at 9 days of age, which is the average age of the tumor used for these studies. This indicates that the dividing cells were arrested, and had accumulated at the rate at which these cells normally divide. Differential counts made on the total number of cells in mitosis show that the arrest is at metaphase. The results of these experiments lend further support to Heilbrunn's hypothesis that the completion of mitosis is dependent on protoplasmic gelation.

Summary

Chemical extraction of fibroblastic carcinomas of the human breast, benign mammary lesions, a normal breast, and a medullary carcinoma yielded a nondialyzable, water-soluble substance, exhibiting several positive reactions for sugars.

The tissue extracts from the cases of fibroblastic carcinomas were found to reduce protoplasmic viscosity and to arrest mitosis of Ehrlich ascites tumor cells in metaphase. Extracts from the other breast lesions were inactive in this respect.

These findings give further support to the theory that completion of mitosis is dependent on cytoplasmic gelation.

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Chemical and Pathological Studies on Aortic Atherosclerosis

A Comparative Study of One Hundred Twenty-Eight Aortas in South African Bantu and White Subjects

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Introduction

The rarity of myocardial infarction among the hospitalized South African Bantu has made them a suitable community for studying the geographical pathology of atherosclerosis and coronary heart disease. It is generally accepted that myocardial infarction is rare in the Bantu,[1-4] but there is less certainty regarding the incidence of severe atherosclerosis. While atherosclerosis is widespread, it has been reported that in the older age groups severe atherosclerosis is less frequent than in Denmark and North America,[1,4] but this has recently been denied by Laurie and Woods.[5]

Since our earlier observations on the severity of atherosclerosis were subjective, we have attempted to determine whether these racial differences could be demonstrated more objectively by chemical analysis. Earlier studies, especially on the lipid fractions, indicated the feasibility of doing so in population groups, if not in individuals.[6,7] At the same time it was hoped that further information would be obtained on the value of this method in grading.

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Accordingly, the dry weight, ash, and calcium, total fat, cholesterol, phospholipid, total nitrogen, elastin, collagen, and hexosamine concentrations were determined in aortas from Bantu and white subjects. While the extent of our study was limited and our series is small, it appears worth recording, since our findings indicate that racial differences in the chemical composition of the aorta do exist. They also cast some light on the value of these methods as a means of objective grading of atherosclerosis.

Materials and Methods

Source of Aortas.—The aortas from Bantu subjects were obtained from consecutive autopsies at Baragwanath Hospital, and those from white subjects, at the Johannesburg General Hospital. To increase numbers in certain age groups poorly represented in the hospital material, unselected aortas were also obtained from the Medico-Legal Laboratories in Johannesburg. All aortas were unselected except that cases of syphilitic aortitis were excluded. Altogether, 70 aortas from Bantu subjects and 58 from white subjects were available for study.

The unfixed aortas reached the laboratory within four hours of removal from cadavers. They were then graded and photographed. After stripping the adventitia and trimming, blocks for histology were taken through the severest lesions in the arch and descending and abdominal portions. This also permitted a check on the adequate removal of the tunica adventitia.

A. Chemical Analysis

The aortas were cut up into portions about 0.5 cm. square and dried for 24 hours approximately in a vacuum desiccator over concentrated sulfuric

acid. This procedure removed all but about 5% moisture, the residual figure being determined on a separate small sample of tissue dried to constant weight at 100 C. The vacuum-dried material was ground in a Willey Mill, using 20-mesh sieve, and stored in airtight receptacles in a cool place.

Ash and Calcium.—Ash and calcium were determined as described by McCance and Shipp.[8]

Total Lipid.—From 200-400 mg. of dry tissue was extracted with Bloor's mixture (alcohol-ether, 8:3) for 10 hours in a Soxhlet apparatus. The solvent was removed and the lipids extracted with petroleum ether (petroleum benzin), at 60-80 C. The latter was evaporated off, and lipids were dried to constant weight at 70-80 C. Duplicate analyses agreed to within 2%.

Phospholipid.—The method of King[9] was employed. Duplicates agreed within 1%.

Cholesterol.—From 50-100 mg. of tissue was digested for about three hours with 0.2 NaOH. The mixture was extracted three to four times with alcohol-acetone (1:1). The supernatant liquors were decanted off and made up to volume (25-100 ml.). Cholesterol was determined by the Liebermann-Burchardt color reaction, as described by King.[9] Duplicate analyses agreed to within 3%.

Total Nitrogen.—This constituent was determined by a micro-Kjeldahl method, as described by Pregl.[10]

Collagen and Elastin.—One hundred milligrams of tissue was defatted with 100 ml. of alcohol-acetone (1:1). The supernatant was removed by decantation and autoclaved with 5 ml. of distilled water in 20 ml. test tubes with raw cotton (cotton wool) for three hours at 25 lb. pressure. This procedure was repeated three times. The supernatant fluids were then poured off, pooled, dried in an air oven at 80 C, and evaporated to dryness. This operation converts the collagen to gelatin but leaves the elastin undissolved. Both elastin and gelatin fractions were hydrolyzed separately with 2 ml. of 6 N HCl for six hours at 25 lb. pressure. Hydrolysates were neutralized with 6 N NaOH, filtered, and bulked (usually to 25 ml.). The amounts of elastin and collagen were determined by liberation of hydroxyproline, as described by Neuman and Logan,[11] the conversion factors being 7.46 for collagen and 52.3 for elastin. Ox hide, highly purified (95% collagen), was used as a control and hydrolyzed simultaneously with each batch of samples. Recoveries varied between 96% and 101%. Duplicate analysis showed agreement within 5% for collagen and 7% for elastin.

Hexosamine.—From 30-50 mg. of tissue was hydrolyzed with 4 N HCl for 15 hours in glass-stoppered flasks (25 ml.). Hydrolysates were treated with Dowex-50 resin (an ion-exchange resin), as described by Boas,[12] to separate off amino acids and sugars, which interfere with the color reaction. After elution of the hexosamines, the color was developed by the method of Elson Morgan, as modified by Boas.[12] Destruction of added glucosamine during hydrolysis averaged 6.2%. Mean recovery of glucosamine standard treated with the resin was 90.4%. All results are presented as percentages of dry weight.

B. Grading of Atherosclerotic Lesions

The aortas were divided into four segments, and each was graded on naked-eye appearance according to the degree of atherosclerosis by one worker (J. H.), wholly unaware of the results of the chemical analysis, as follows:

0: No lesion (histological section, however, often showing slight intimal lesions in such cases)
1: Minimal scattered lesions, linear or patchy, whether fatty, mucinous, or fibrous
2: More advanced lesions with definite plaque formation
3: Plaques large and confluent and covering greater part of intimal surface, palpation and section sometimes showing slight calcification in such cases not visible to the naked eye
4: Definite ulceration and/or calcification; widespread involvement of the intimal surface usually present
5: The severest degree of atheroma with marked ulceration and calcification (arteritis deformans)

The values for all segments were summated, and a final grading of 0 to 5 was adopted. Representative aortas for each grade are shown in Figure 1.

This grading is subjective, and we would now prefer to use the more satisfactory classification proposed by Gore and Tejada.[13] Furthermore, since the aortas were destroyed in the chemical analysis, grading could not be carried out simultaneously, although comparison of photographs did permit some check (admittedly inferior) on the consistency of our grading. We consider that in future studies the vessel should be bisected longitudinally and only one-half used for chemical analysis, the other being retained for later grading. Further, it would have been preferable to have analyzed each segment separately, so that errors introduced by the summation of segments with wide variations could have been avoided. This error may partly explain the wide variation shown in some components. Finally, colored photographs would have been preferable to black-and-white prints.

Results

Effect of Sampling Errors.—Apart from subjective grading, the major source of inaccuracy in making comparisons between

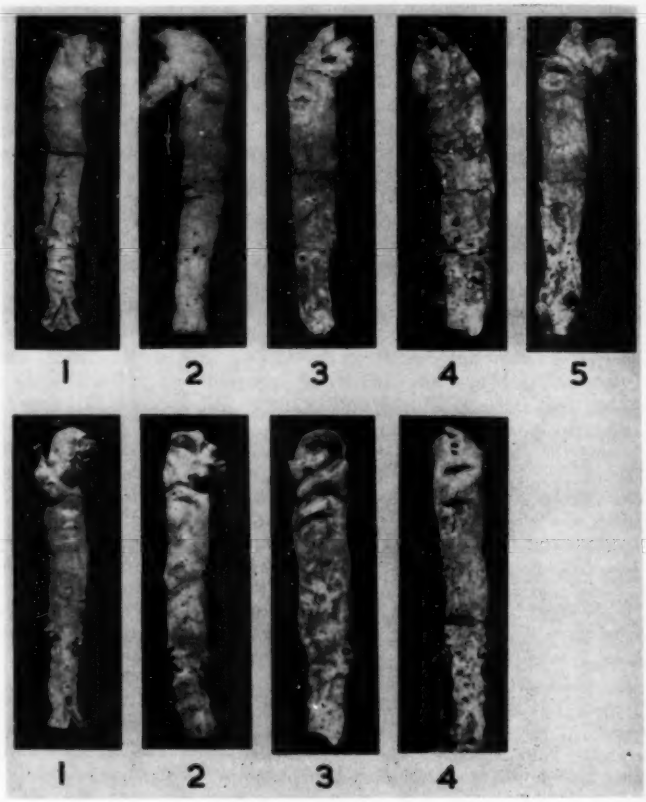

Fig. 1.—Composite photograph showing typical aortas in each grade from both races. The vessels from white subjects are in the upper group; the aortas from Bantu subjects are in the lower group.

Bantu and white subjects is a failure to use completely comparable samples from the two populations. Although we believe that our samples are free from intentional bias in regard to atherosclerosis, our numbers are small, and this possibility cannot be excluded with certainty. Despite these inadequacies, the differences demonstrated in certain chemical components are of sufficient magnitude and constancy as not to be readily explainable by sampling error, and almost certainly represent true differences between the two races. Moreover, the similarities in other chemical components support this view.

All statistical remarks are subject to the basic assumption that the autopsies are a random and representative sample of both the Bantu and the white populations. The calculations have been made according to methods given by Hald.[14] Unless stated otherwise, the term "significant" is used at the 95% level.

Effect of Sex.—In general, males and females showed similar trends in each racial group. Moreover, the average value in each grade and for corresponding age in each sex did not seem to vary significantly apart from weight. It was therefore decided to combine male and female data for all components except that of weight.

I. Grade of Atherosclerosis and Chemical Composition

The question whether chemical analysis provides a satisfactory objective method for determining the degree of atherosclerosis is complicated by two factors. First, since

the grading is subjective, we cannot be completely certain that grading was identical in the two races. Second, the grading is not equidistant; i. e., an aorta classified as Grade 5 is not necessarily five times as severely involved as an aorta classified as Grade 1. This excludes the possibility of curve fitting, as has been undertaken with the analysis in respect to ages (*vide infra*). The most effective analysis obtainable is made by comparing averages between successive grades for each race, and also by comparing the averages for the same grade in the two races.

Dry Weight.—The average weight increases significantly with grade in both sexes and races (Table 1), but the difference between races is not significant for the same grade. Unfortunately, the possible value of dry weight as an objective measurement of grade was not appreciated originally, since it was intended to express our findings as percentage composition, and corrections for height of patient and size of histology blocks were not made. The differences between grades, however, appear too great to be explained by such errors.

Ash.—Ash tends to increase with grade for both races. However, in the same grade, ash values between white and Bantu subjects for Grades 3 and 4 show significant differences (Table 1).

Calcium.—Grading with respect to calcium concentration shows striking differences between Bantu and white subjects. Among Bantu subjects there are some differences between Grades 0 and 1 and Grades 2, 3, and 4, but no clear trend is apparent. Calcium values for white subjects, however, show a very clear trend with increasing grade; Table 2 gives the 95% confidence limits for the average within each grade. The marked overlap of these limits should be noted, indicating that while the grades can establish a calcium trend, there is a tremendous variation of calcium values within each grade. The differences between grades (white subjects only) were all found to be significant except for Grades 1 and 2.

In the higher grades (3 and 4) the mean calcium concentration for the same grade differs significantly between Bantu and white subjects; and the higher the grade the more marked does this divergence become (Table 1).

Total Lipid.—Average total lipid values tend to increase with grade. Differences in values between successive grades for the Bantu are significant except for Grades 1 and 2. With white subjects the values for each grade are significantly different except for Grades 2 and 3. While differences between races for the same grade are not significant, in general, the values for the white subjects are higher than those for the Bantu, except for Grade 1 (Table 1).

Cholesterol.—The trend for cholesterol to increase with grade is clearly shown in Table 1, but there was an immense overlap of observations between the grades. Hence, grading with respect to cholesterol, as with calcium, makes it possible to establish a trend for group data but is unsatisfactory in any particular case. In Table 3, the 95% confidence limits for the average within each grade are given for both white and Bantu subjects, and it will be noted that these limits are wide. The same table shows the results of testing for a difference between the average of successive grades within each racial group. In the case of the white subjects the values were all significant; in the Bantu, only the differences between Grades 0 and 1 were not significant.

The differences in the averages between the races for the same grade were found not to be significant. In all cases, however, the average cholesterol value of the white subjects was higher than that of the Bantu, and the higher the grade the more marked did this difference become.

Phospholipid.—The tendency for phospholipid to increase with grade is unmistakable, but the difference between grades was not significant in either race, nor were the

TABLE 1.—*Changes in the Mean Chemical Composition*

Grade of Atherosclerosis	No. of Subjects				Dry Weight Aorta, Gm.				Ash †		Calcium †	
	Males		Females		Males		Females					
	B	W	B	W	B	W	B	W	B	W	B	W
0	2	0	0	0	5.97	--	--	--	3.03	--	0.70	--
1	13	1	4	1	6.99	5.08	7.23	7.04	3.44	3.60	0.96	1.39
2	10	11	14	5	8.47	8.83	6.98	6.03	4.86	4.24	1.38	1.51
3	14	8	6	5	9.24	9.74	9.22	8.31	5.08	7.59	1.85	2.76
4	3	5	4	13	12.22	13.03	9.61	11.21	5.46	10.75	1.53	4.12
5	0	6	0	4	--	18.36	--	16.01	--	17.65	--	6.81

* B = Bantu subjects; W, white subjects.
† Expressed as grams per 100 gm. dry weight.

differences between the racial groups for the same grade significant.

Total Nitrogen.—A fairly clear decline in nitrogen concentration occurs with increasing grade. The trend is apparent for the Bantu, but differences between successive grades are not significant. With white subjects, only the difference between Grade 4 and Grade 5 was significant, although that between Grade 3 and Grade 4 was fairly marked. When comparing values between Bantu and white subjects for the same grade, white-subject values are consistently lower than values for the Bantu (Table 1).

TABLE 2.—*Range and Mean Values for Calcium in Each Grade in White Aortas*

Grade	$\bar{x}$	$s/\sqrt{n}$	95% Limits of Confidence	Significance Between Grades
1	1.39	0.505	0.38-2.40	N. S.
2	1.51	0.227	1.06-1.97	$P<0.05$
3	2.76	0.384	1.99-3.53	$P<0.05$
4	4.12	0.463	3.19-5.05	$P<0.05$
5	6.81	1.041	4.73-8.89	

Collagen.—There is an obvious decline of average collagen values with grade; grading reveals a similar picture for the two races (Table 1).

Elastin.—No clear trend with grade was discernible. The average elastin value seems to be slightly lower for white than for Bantu subjects except for Grade 4, but the differences do not appear to be very marked.

Hexosamine.—The average values per grade are shown in Table 1, and no clear trend is apparent.

Summary of Changes in Chemical Composition and Pathological Grading

In each grade, the values for any single chemical component show a wide variation not only between races but for the same racial group. On the other hand, the mean values for certain constituents show a definite correlation with degree of atherosclerosis. This correlation is most readily demonstrable for total weight, percentage of total lipid, cholesterol, and phospholipid,

TABLE 3.—*Mean Values of Cholesterol* and Grading in White-Subject and Bantu Aortas*

Grade	White Subject Aortas			Bantu Aortas		
	$\bar{x}$	95% Confidence Limits	Significance Between Grades	$\bar{x}$	95% Confidence Limits	Significance Between Grades
0	/	/ /		1.05	0.83-1.27	N. S.
1	1.50	1.06-1.94	$P<0.05$	1.41	1.14-1.68	$P<0.05$
2	2.41	1.93-2.89	$P<0.05$	2.31	1.94-2.68	$P<0.05$
3	4.40	3.47-5.33	$P<0.05$	3.60	3.01-4.19	$P<0.05$
4	6.15	5.39-6.91	$P<0.05$	4.55	3.87-5.23	
5	8.47	6.74-10.20		/	/ /	

* Expressed as grams per 100 gm. dry weight.

*of the Aorta in Relation to Degree of Atherosclerosis**

Total Lipid		Cholesterol †		Phospholipid †		Total Nitrogen †		Collagen †		Elastin †		Hexosamine †	
B	W	B	W	B	W	B	W	B	W	B	W	B	W
11.3	--	1.05	--	2.19	--	14.3	--	26.1	--	30.8	--	0.86	--
13.7	12.3	1.41	1.50	2.32	1.73	14.8	14.5	23.0	25.3	29.4	26.3	0.80	0.74
14.9	16.6	2.31	2.41	2.84	2.87	14.3	13.7	22.8	22.5	30.2	28.9	0.92	0.83
16.9	18.4	3.60	4.40	3.12	3.56	13.8	13.1	21.2	20.4	30.8	27.3	0.89	0.86
19.4	22.5	4.55	6.15	3.17	3.65	13.6	12.3	21.0	21.8	27.7	28.3	0.87	0.80
--	27.7	--	8.47	--	4.04	--	10.1	--	20.2	--	21.2	--	0.60

in both races, as well as for ash and calcium in white subjects. These findings are in agreement with the experiences of previous workers.[6,7,15-20] Other constituents show less prominent differences, although they might have proved of significance had the number of aortas studied been greater.

There appears to be no great difference for the two races up to and including Grade 3 in respect of dry weight, total lipid, and phospholipid. However, with respect to ash, calcium, and cholesterol, certain obvious differences are apparent after Grade 2 which we believe represent qualitative differences in the atherosclerotic process in the two races. Accordingly, while it may be possible to utilize chemical analysis for grading atherosclerosis for group data within one racial group, it would appear inadvisable to use components such as calcium and cholesterol as accurate indices of atherosclerosis in different races. It should be noted that such a simple measurement as dry weight may prove as satisfactory an estimate of degree of atherosclerosis as the more complicated determinations.

The significance of these qualitative differences in the aorta in relation to the complications of atherosclerosis in the smaller vessels requires investigation, as the correlation between the severity of atherosclerosis in the aorta and coronary arteries is not complete.[21]

II. Age Trends in Atherosclerosis and Chemical Composition

Grade of Atherosclerosis.—The mean grade of atherosclerosis increases with age in both races (Table 4), and severe atherosclerosis tends to be less severe in Bantu than in white subjects in the older age groups. In view of our observations on chemical analysis and grading, this is unlikely to be due entirely to subjective grading error.

Chemical Changes.—While changes in chemical composition with age may be mainly dependent on the increasing severity of atherosclerosis with age, a further factor must be considered. There were sufficient aortas available of Grade 2 severity to permit comparison of chemical analysis below and above 45 years of age in both races.

TABLE 4.—*Mean Average Grade of Atherosclerosis in Each Age Group for Bantu and White Subjects*

	Bantu					White				
Age Range, Yr.	Males		Females		Both Sexes	Males		Females		Both Sexes
25-34	0.7	(9) *	1.7	(5)	1.0	1.8	(5)	2.0	(1)	1.9
35-44	2.0	(7)	2.0	(4)	2.0	2.0	(3)	2.0	(2)	2.0
45-54	2.2	(8)	1.3	(3)	1.9	2.5	(5)	2.3	(5)	2.4
55-64	2.4	(5)	2.8	(8)	2.5	3.4	(9)	3.4	(5)	3.4
65-74	2.3	(6)	2.6	(5)	2.4	3.4	(4)	3.8	(9)	3.7
75+	3.1	(7)	2.8	(3)	3.1	4.3	(4)	4.1	(6)	4.2

* The figures in parentheses represent the number of aortas.

TABLE 5.—*Changes in Composition in Aortas with Atherosclerosis of Grade 2 According to Age*

	Bantu Aortas			White Aortas		
	Under 45 (9)* (a)	Over 45(15)* (b)	Significance Between (a) & (b)	Under 45 (7)* (c)	Over 45 (8)* (d)	Significance Between (c) & (d)
Dry Weight, gm.						
Males	6.39	9.36	$P<0.01$	6.49	11.65	$P<0.01$
Females	4.88	8.55	$P<0.01$	5.31	7.10	--
Ash †	3.60	5.61	$P<0.05$	2.95	5.83	$P<0.01$
Calcium †	1.01	1.60	N. S.	0.91	2.29	$P<0.01$
Total Lipid †	13.9	15.5	N. S.	14.5	19.3	$P<0.01$
Cholesterol †	1.42	2.84	$P<0.01$	1.83	3.16	$P<0.01$
Phospholipid †	2.54	3.04	N. S.	2.68	3.11	N. S.
Total Nitrogen †	14.6	14.1	N. S.	14.1	13.3	N. S.
Collagen †	24.6	21.7	$P<0.05$	24.3	21.5	N. S.
Elastin †	27.5	31.8	$P<0.05$	29.2	28.4	N. S.
Hexosamine †	0.88	0.95	N. S.	0.79	0.89	N. S.

* The figures in parentheses refer to the number of aortas in each group.
† Expressed as grams per 100 gm. dry weight.

These findings are presented in Table 5 and show that for certain constituents significant chemical changes occur with age within the same grade. This observation would indicate that our figures accordingly represent a summation of both aging and atherosclerotic processes, as shown by Haythorn et al.,[18] thus complicating the use of such measurements for objective grading.

Dry Weight.—In both racial groups there is a steady increase in the weight of the aorta with age, confirming observations of previous workers [16,17] (Table 6 and Fig. 2). The increase is, however, less in Bantu than in white subjects after 45 years, and differences between Bantu men and women are less pronounced than between the white sexes.

Ash.—Table 6 and Figure 3 confirm earlier reports [16] in demonstrating an increase in percentage of ash with age, but whereas in the Bantu the mean ash concentration is only doubled in the 75+-year age group as compared with the 25- to 34-year groups, the corresponding increase is fivefold in white aortas.

Calcium.—The changes in calcium concentration follow those in ash (Table 6, Fig. 3) and are in accord with the findings of previous workers.[15-19] This element provides the most striking differences between the two races. From the age of 30 years onward the Bantu show a slow linear increase (Fig. 3), but the data on the aortas of the white subjects can be represented only by a straight line from the age of 40

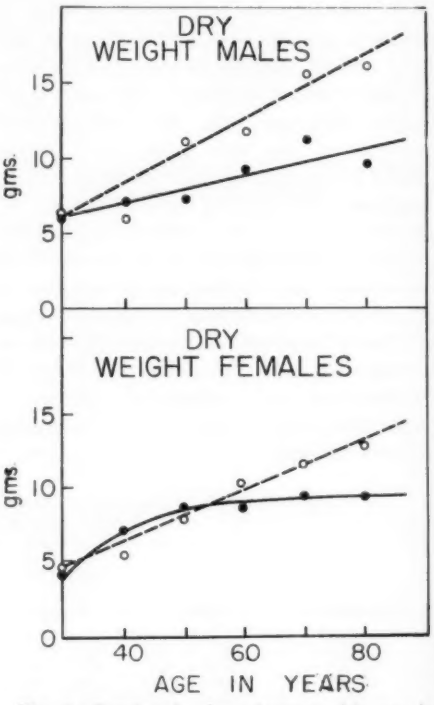

Fig. 2.—Graphs showing changes with age in mean dry weight of aortas for each race. Values for Bantu subjects are expressed by solid line and circles; values for white subjects, by broken line and clear circles.

years onward. The estimated slope of Bantu data after 40 years is 0.021; the corresponding figure for white subjects is 0.122; these differences are statistically significant at the 99% level (u=3.48). In the case of the Bantu, constant variance is assumed, an assumption which is just passable. With white subjects this convenient assumption is no longer possible, but as the standard deviation increased with age, use is made of the hypothesis S. D.=0.055× age-1.550. The equations of the Bantu and white lines are Ca=1.41+0.021 (age-53.7) and Ca=2.33+0.122 (age-50.5), respectively. These racial differences demonstrated above show that the changes in calcium concentration are not alone the result of degree of atherosclerosis or of age.

Total Lipid.—As can be seen from Table 6 and Figure 4, average total lipids in the aorta show a rough linear increase with age, as reported by others. Assuming constant variance for both Bantu and white subjects, slopes were found to be 0.10 and 0.20, respectively. This difference just fails to be significant at the 95% level.

Cholesterol.—In white subjects, cholesterol rises with age in an almost linear fashion (Fig. 4).[6,16,20] With the Bantu, in contrast, cholesterol rises to a maximum between 60 and 70 years and thereafter seems to decrease; the behavior of the data is well represented by curve *b* in Figure 4. If readings taken at the age of 70 and 80 years are ignored, the first four points conceivably form a straight line as given by line *a* in Figure 4. If line *a* is not accepted as being a reasonable representation of the data, we can conclude that the Bantu in the older age groups differ greatly from similar white subjects. Differences between average cholesterol concentrations of Bantu and white subjects "65 to 74" and "75+ years" were tested, and were found to be significant.

Correlation Between Cholesterol and Calcium.—In white subjects, a fairly good linear relationship is apparent between calcium and cholesterol for values less than 7.0 gm. % dry weight. A quadratic curve probably gives a fair representation of the relationship. Correlation values of 0.64 and 0.45 were obtained between calcium and cholesterol for white and Bantu subjects. The Bantu would be expected to be lower because of the curved trend of their cholesterol concentration with age.

Phospholipid.—There is a curved increase in phospholipid with age, as noted by Buck and Rossiter.[20] For both racial groups, a maximum value is reached at about 50 years. At 30 years, values for the two races seem to be identical; thereafter the value for white subjects seems to exceed the Bantu value by a constant amount from about the age of 40 years onward (Table 6 and Fig. 4).

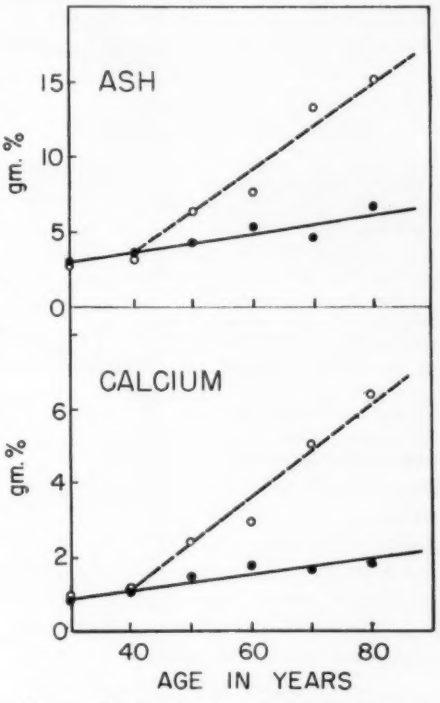

Fig. 3.—Graphs showing changes with age in mean ash and calcium concentrations in aortas for the two sexes combined (grams per 100 gm. dry weight). Values for Bantu subjects are expressed by solid line and circles; values for white subjects, by broken line and clear circles.

TABLE 6.—*Changes in the Mean Chemical*

Age Group	Dry Weight, Gm.				Ash †		Calcium †		Total Lipid †	
	Males		Females							
	B	W	B	W	B	W	B	W	B	W
25-34	6.27	6.52	4.43	4.71	3.00	2.92	0.82	0.86	12.5	13.6
35-44	7.26	6.12	7.25	5.61	3.00	3.20	1.03	1.08	15.1	16.3
45-54	7.46	11.35	8.52	7.95	4.40	6.43	1.48	2.40	15.3	20.6
55-64	9.50	12.12	8.61	10.31	5.39	7.70	1.77	2.97	16.2	20.0
65-74	11.00	16.9	9.44	11.55	4.76	13.53	1.68	5.06	17.6	24.5
75+	9.93	16.46	9.33	12.88	6.83	15.32	1.81	6.21	17.5	23.3

* B=bantu aortas; W, white aortas.
† Expressed as grams per 100 gm. dry weight.

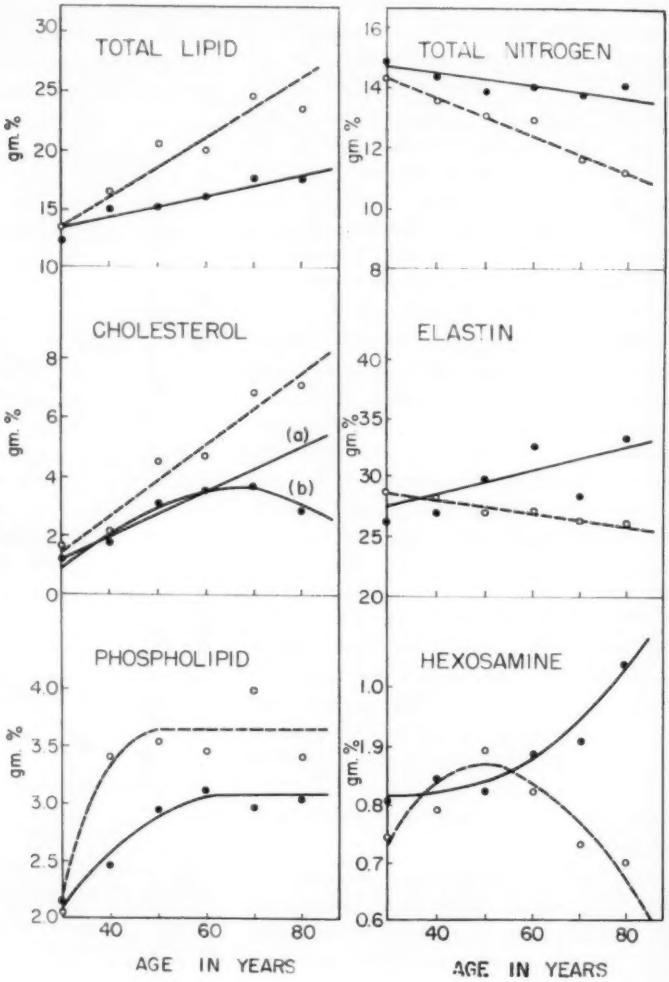

Fig. 4.—Graphs showing changes with age in mean total lipid, cholesterol, phospholipid, total nitrogen, elastin, and hexosamine concentrations for the two sexes combined (grams per 100 gm. dry weight). Values for Bantu subjects are expressed by solid line and circles; values for white subjects, by broken line and clear circles.

Composition of the Aorta in Relation to Age Group*

Cholesterol †		Phospholipid †		Total Nitrogen †		Collagen †		Elastin †		Hexosamine †	
B	W	B	W	B	W	B	W	B	W	B	W
1.22	1.66	2.16	2.05	14.9	14.4	23.9	23.9	26.4	28.8	0.81	0.75
1.78	2.12	2.48	3.41	14.4	13.6	24.1	21.8	27.1	28.3	0.84	0.79
3.08	4.56	2.96	3.54	13.9	13.1	20.9	20.5	29.8	27.0	0.82	0.90
3.54	4.72	3.13	3.46	14.0	12.9	20.2	23.5	32.6	27.2	0.89	0.82
3.68	6.86	2.98	4.01	13.7	11.6	23.0	19.4	28.3	26.3	0.91	0.73
2.83	7.10	3.05	3.42	14.1	11.1	21.6	20.9	33.2	38.0	1.06	0.70

Total Nitrogen.—White subjects show a linear decrease with age (Table 6, Fig. 4). The Bantu data do not give such a satisfactory linear representation, but to make comparison possible a straight line was fitted to the Bantu data as well. The difference in slopes of the lines, 0.019 and 0.066, was found to be significant (u=3.36). The assumption of constant variance was reasonable for both racial groups.

Collagen.—There seems to be no racial difference with respect to collagen concentrations, and there is a suggestion that collagen decreases with age, which is contrary to the findings of Faber and Møller-Hou[22] and Myers and Lang.[23] The data, however, are highly variable, and very much larger groups require to be investigated in order to establish a clear trend (Table 6).

Elastin.—The Bantu data are variable (Fig. 4), in contrast to the white-subject data; but the trends are unmistakable, and are well represented by straight lines. These were tested for slope (as being representative of the trend) and found to be statistically significant at the 99% level (u=4.1). With white subjects, the variance obviously increases with age. In the latter, a very fair assumption was S. D.=0.073+0.284. The white-subject line was found to be elastin=27.56−0.058 (age-49.7). The equation of the Bantu line was found to be elastin=29.80+0.02 (age-53.7). The white-subject data confirm observations made in the studies of others.[22,23]

Hexosamine.—Figure 4 clearly shows that the data on the two racial groups are radically different with respect to hexosamine. The Bantu show a steady curved increase with age, while the white subjects show a curved increase, reaching a maximum at the age of 50, with a steep decline thereafter. These curves are so different that no further statistical analysis was carried out. These values were determined to give indication of proportion of acid mucopolysaccharide present. Faber used the proportion of sulfate to give indication of chondroitin sulfate, and the above observations for white subjects are in accord with his results.[17]

Histological Examination

It was soon apparent that histological examination was of little value for purposes of grading, thereby confirming the view of Rosenthal.[6] Observations, however, provided some check on the type of lesion present, and the studies confirmed reasonably well the naked-eye appearance of the plaques.

Comment

With increasing age, there are distinct differences both in the severity of atherosclerosis and in the chemical composition of aortas from Bantu and white subjects. Assuming that the aortas constituted comparable samples, our results support the view that severe aortic atherosclerosis in the older age groups is less common in Bantu than in white subjects.

The picture, however, is obscured by the fact that the chemical changes are the result both of atherosclerosis and of age, and further studies are indicated to separate

the effect of each factor. Allowing that part of the differences is due to atherosclerosis, it would still appear that the differences demonstrated are insufficient to explain the markedly different incidence in myocardial infarction and coronary occlusion in the two races if these are a direct function of the severity of atherosclerosis. It is feasible that the correlation between the severity of atherosclerosis in the coronary arteries and that in the aorta varies in different races, but we have no further information on this possibility.

It is usual to correlate the severity of the atherosclerotic process with its complications, notably ulceration and thrombosis; but the present findings indicate that qualitative changes in the atherosclerotic and aging processes may also be considered. For example, the severity of atherosclerosis in two aortas may appear similar not only on gross examination but on cholesterol and weight measurements, whereas the quantity of calcium may be very different. We are unwilling as yet to assess the etiological significance of these qualitative changes within aortas of the same grade, but they may eventually prove of importance in indicating causal factors.

In conclusion, it would seem that while grading by chemical analysis may be of value in one racial group of similar age, its use in regard to different races and different age groups is more doubtful. Furthermore, the simple measurement of dry weight may be as satisfactory as more complicated procedures. Since, however, the use of subjective grading by different authors in different countries and at different times will tend to obscure the relationship of severe atherosclerosis and myocardial infarction, the need of developing objective methods further is clear.[25]

Summary

The dry weight and concentrations of total ash, calcium, total lipid, cholesterol, phospholipid, nitrogen, elastin, collagen, and hexosamine were determined in the aortas from 70 Bantu and 58 white subjects, the aortas being previously graded for the degree of atherosclerosis.

Mean total weight, and lipid, cholesterol, and phospholipid concentrations showed definite correlation with the degree of atherosclerosis in both races. Ash and calcium concentrations showed a correlation in white but not in Bantu aortas. Furthermore, in aortas of the same grade there were differences between the two races in regard to ash, calcium, and cholesterol concentrations. The differences were sufficiently large as to be not wholly explicable on the basis of subjective error in grading, and hence probably represent true qualitative differences in atherosclerosis, especially in regard to calcification. However, there is evidence that considerable changes in chemical composition also occur with age within the same grade, thus obscuring the picture.

Differences in the concentration of various chemical components, notably calcium, cholesterol, and hexosamine, were demonstrated between Bantu and white subjects, especially in the older decades. Further, the mean degree of atherosclerosis and total dry weight were significantly less in Bantu than in white subjects. It is suggested, accordingly, that further racial studies should take into consideration qualitative as well as quantitative differences in the atherosclerotic process. However, these racial differences, if representative of the degree of atherosclerosis and if equally applicable to the coronary artery system, would appear insufficient to explain the marked differences in the incidence of myocardial infarction demonstrated between the two races.

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Production of an Experimental Ulcerative "Colitis" in Rabbits

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Introduction

The pathogenesis of ulcerative colitis probably involves more than one cause.[1] Investigation of each possible mechanism thus should be useful in fully clarifying the nature of the disease. Development of an experimental colitis especially would enhance the study of the disorder. Abnormalities in the colon of animals have been induced by deficiency of folic acid,[2] by administration of lysozyme[3] or histamine,[4] by the injection of Staphylococcus toxin,[5] by mesenteric lymphatic obstruction,[6] and by the injection of cholinergic compounds—acetylcholine,[7] carbachol (Doryl), methacholine (Mecholyl),[8] and neostigmine (Prostigmin). However, the significance of the "colitis" induced under these conditions has been difficult to evaluate. The participation of a hypersensitivity mechanism in the evolution of ulcerative colitis has presented an intriguing possibility. Pathological immune complications, such as reactions to drugs and blood transfusions, erythema nodosum, iritis, hemolytic anemia, and the development of an increased γ-globulin, including a τ-globulin, are not rare during the course of ulcerative colitis.[9] The occasional history of rheumatic fever and allergic diseases in patients with ulcerative colitis and the association of ulcerative colitis with hypersensitivity reactions,[10] periarteritis nodosa, allergic vasculitis, lupus erythematosus, purpura, scleroderma,[11-14] and rheumatic fever, albeit infrequent, would not be incompatible with an immunological pathogenesis, at least in some cases. The disorder might involve some type of antigen-antibody reaction, possibly a form of autoimmunization. It seemed of interest, therefore, to ascertain the response of the colon of rabbits to experimentally induced conditions of tissue hypersensitivity.

Previous Observations.—Sensitization of the digestive tract has been accomplished infrequently in the past. Successful, but limited, efforts include the Arthus reaction in the stomach[15,16] and in an exteriorized segment of colon,[17] active and passive sensitization of the stomach,[18,19] and passive local sensitization of the ileum, cecum, and colon by the injection of human serum containing atopic reagins.[18,20,21] The administration of toxins from Gram-negative bacteria to rabbits previously given cholera bacilli resulted in hemorrhagic, necrotizing lesions of the small intestine,[22] subsequently identified with the Shwartzman phenomenon.

Initial studies in our laboratory dealt with the possibility of producing anti-dog-colon antibodies by the repeated injection of lyophilized dog colon mucosa into rabbits, dog serum containing autoantibodies against dog colon, and rabbit serum containing autoantibodies against rabbit colon, as had been reported in experimental glomerulonephritis.[23,24] Preliminary experiments with normal tissue and with colon from animals who had received methacholine injected alone, and with Freund's adjuvant[25] or with Staphylococcus toxin,[26] to increase the

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antigenicity of the preparation, failed to induce specific complement-fixing antibodies. Intradermal tests with extracts of lyophilized colon mucosa in patients with ulcerative colitis also were negative. It was possible, however, to reproduce the Arthus reaction[27-29] in rabbits sensitized to egg albumin by the injection of 0.2 ml. of a 0.5% solution of egg albumin, subserosally at one or multiple sites in the colon and the small intestine, at abdominal operation. The typical Shwartzman reaction[30-32] also could be induced in multiple sites of the colon and small intestine and, concurrently, in the skin, by initial injections of 0.25 ml. of a 1:10 dilution of lysed Serratia marcescens culture, followed 24 hours later by 1 ml. of a 1:2 dilution of the same lysate given intravenously. Positive dermal responses developed within three hours. The colon, examined after seven hours, contained severe hemorrhagic and necrotizing lesions, localized to the sites of injection. The results thus suggested that the colon, at least in rabbits, was capable of responding to hypersensitivity mechanisms.

Auer Procedure.—There appeared to be at least two major problems in the production of "hypersensitivity colitis": adequate sensitization of the animals, and a method of localizing a cytotoxic antigen-antibody reaction in the colon. The Auer procedure[33] seemed appropriate for these purposes. According to Auer, a sensitized animal, under proper conditions, could localize the sensitizing antigen in inflamed tissues and thereby produce the state necessary for a local hypersensitivity or anaphylactic response. The phenomenon thus involves a state of mild nonspecific irritation of the tissue area under study, at the time of systemic administration of antigen to an animal already sensitized to the specific antigen. Auer demonstrated this effect in the ears of rabbits sensitized to horse serum by the local application of xylene. Opie[34] obtained similar results with egg albumin as the antigen and hot water as the tissue irritant. Menkin[35] confirmed Auer's findings, demonstrated the attraction of inflamed tissue for circulating antigen and antibody, and emphasized the increased capillary permeability in sites of inflammation, an important condition for the local concentration of antigen and antibody, as noted also by other investigators.[36,37] Walsh and Cannon,[38] investigating immunization of the respiratory mucosa, found increased regional antibody formation after the preliminary local instillation of a mild irritant (0.2 to 1.0 ml. of a 1% or 1.5% solution of formalin). Antibody titers of the inflamed area of regional immunization were consistently higher than in other tissues tested. Fox[39] had noted previously the localization and concentration of antibodies in areas of inflammation after intravenous administration of antibody-containing sera. Hopps,[40] using the Auer principle, apparently demonstrated a local antigen-antibody reaction in experimental wounds in rabbits.

Theoretical Basis for Study.—The preceding observations suggested the possibility of producing an experimental colitis in rabbits sensitized with a soluble antigen, egg albumin; the subsequent antibody response presumably would be similarly generalized. Rectal instillation of a mild, nonspecific irritant would induce slight inflammation and edema locally and, with the accompanying increased capillary permeability, might facilitate local accumulation of previously formed antibody and of newly injected specific antigen. The combination of antigen and antibody in the mildly inflamed colon then might produce a more pronounced inflammatory response, "colitis."

Methods of Study Materials

The experiments were performed in adult albino male and female rabbits, weighing approximately 2 to 2.5 kg. Studies were made initially on a heterogeneous Swift strain, and later New Zealand Whites exclusively. The animals were maintained on a diet of Purina Rabbit Pellets and water.

The effects of varying dilutions and volumes of formalin upon the rectum and colon were studied initially in more than 25 rabbits. Solutions of 0.5% had no demonstrable action, whereas concentrations of 3% and 4% were too irritating. The standard

irritant consisted of two rectal instillations of 1 ml. of a 1% (occasionally 2%) solution of formalin at intervals of 12 or 24 hours, gently introduced via a rubber catheter, with the aid of an infant proctoscope. Mild to moderate hyperemia frequently became visible within several hours, subsiding within 6 to 25 hours. The rectal mucosa often appeared unchanged grossly, though histologic study usually demonstrated edema and mild cellular infiltration. Six unsensitized rabbits given 1 ml. of formalin (1%) intrarectally for two days every two weeks during a period of six months demonstrated only mild transient hyperemia of the rectal mucosa proctoscopically and no significant changes at autopsy.

In the earlier studies, rabbits were sensitized with four-times-recrystallized egg albumin powder, prepared according to the method of Kekwick and Cannan.[43]

Egg albumin (Mallinckrodt Chemical Works) and Emulsol egg albumin were used subsequently. Several schedules of sensitization were employed, chiefly the multiple portal procedure, described by Wissler et al.[42] In early experiments sensitization was accomplished by the single intramuscular injection of Freund's adjuvant, 1 ml., together with 10 or 20 mg. of egg albumin. In another program the egg albumin was injected via intradermal, subcutaneous, and intraperitoneal routes on three days of each week (10 mg. of egg albumin daily), with weekly booster doses of 10 or 20 mg. for two additional weeks. A similar schedule consisted of injections via five portals simultaneously on three successive days each week for three weeks (intradermal 0.2 ml.; subcutaneous, intramuscular, intraperitoneal, and intravenous routes 1.2 ml. each, of a 2% solution of egg albumin), followed by weekly intraperitoneal injections for three weeks. A program employed later consisted of injections via three portals (intraperitoneal, first day; intradermal and subcutaneous, second day, and intraperitoneal, third day), preceded by Freund's adjuvant, 1 ml., with 10 to 100 mg. of egg albumin injected subcutaneously or intramuscularly. The degrees of sensitization achieved with the various modifications, as judged by the Arthus reaction and the subsequent colon responses to the Auer test, did not appear to differ appreciably. Another procedure consisted in the combination of Freund's adjuvant, 1 ml., together with 10 mg. of egg albumin subcutaneously on the first day, followed by multiple portal injections, 100 mg. of egg albumin being administered daily on two consecutive days during the first week and on three successive days during each of the following two weeks. Since December, 1957, the multiple portal method has been utilized without an adjuvant. On three successive days for three weeks (or less, as indicated by the rabbits' reaction to the intradermal injections, the site of which may become inflamed, and even necrotic, during the last week of sensitization) the rabbits receive 1 ml. of 2% five-times-crystallized Pentex egg albumin * at each of five portals (intravenous, intraperitoneal, intramuscular, intradermal, and subcutaneous)—a total of 5 ml. a day, or 100 mg. of egg albumin. This procedure has produced the most highly sensitized rabbits and the most pronounced "Auer" colon reactions.

Sensitization was indicated by positive Arthus reactions to the intradermal injection of egg albumin (0.1 or 0.2 ml. of a 0.5% solution). A convenient "screening" technique, the Arthus test in the rabbit reflects the presence of significant amounts of specific precipitins. Several investigators[43–45] have found a definite parallelism between the precipitin content of serum and the intensity of the cutaneous reaction. Sensitization also was indicated by the not infrequent occurrence of anaphylaxis, especially when the final antigen was given intravenously; slow injection over a period of 15 or 20 minutes reduced this hazard.[46]

In various experiments, challenging doses of egg albumin in amounts of 5 to 1,000 mg. were injected intraperitoneally, intravenously, or rectally approximately one week after the positive Arthus tests. In the early studies, administration of the challenging antigen rectally produced negative results; positive results were obtained subsequently and are described later. The responses to intraperitoneal and to intravenous administration were approximately the same; however, the intravenous route had the disadvantage of a higher incidence of acute anaphylactic reactions. Intraperitoneal doses of 5 to 15 mg. of antigen were ineffectual; 30 mg. was more effective, and this quantity was employed in most studies. The antigen was given at intervals ranging from 30 minutes before the second rectal instillation of formalin to five hours later. While the responsiveness of the colon did not correlate precisely with the timing of the challenging antigen, as tested in a considerable number of rabbits, the optimal period appeared to be 45 to 120 minutes following the irritant. Proctoscopic observation of the presence of formalin-induced hyperemia was a helpful guide to the appropriate time for the challenging antigen. It seemed likely that premature injection of antigen might result in loss of antigen in the urine or in other tissues before development of the rectal irritation; delayed administration might reduce the likelihood of accumulation of antigen in the bowel because of subsidence of the rectal irritation and the associated vascular permeability.

Observations.—The rabbits remained under careful clinical surveillance and were observed particularly for evidence of diarrhea and rectal

* Studies of this preparation by paper electrophoresis indicate at least 93% purity.

bleeding. The rectum was inspected initially with the aid of an infant proctoscope, introduced to a level 6 cm. above the anal ring; an additional 1 or 2 cm. could be visualized in the absence of fecal material. The proctoscope employed at present permits visualization for a distance of approximately 12 cm. Examinations initially were made at intervals of 2 hours for the first 6 or 8 hours and subsequently at 12, 24, and 48-hour intervals or longer, depending upon the experiment. At present, observations are made once a day between four and six hours after introduction of the antigen, considered the time of maximal colon reaction. Proctoscopy did not necessarily demonstrate the full extent of the lesions when present, but served as a useful guide. More complete anatomic observations were made at elective autopsy after the rabbits had been killed with pentobarbital (Nembutal) sodium injected intravenously. The rectum and colon were removed completely and flushed with isotonic saline. After careful gross inspection, sections were removed at various levels and from abnormal-appearing areas. Tissues were fixed in alcohol-formalin or Bouin's solution and stained with hematoxylin and eosin; the trichrome, elastic-tissue, and mucin stain were used occasionally.

The following studies were undertaken: (*a*) the gross and histologic appearance of the normal rabbit colon and rectum; (*b*) the reaction of the colon in sensitized rabbits to the single Auer procedure, the "acute" response, and (*c*) the reaction of the colon in sensitized rabbits to repeated Auer procedures, the "chronic" response. Control observations were made in sensitized and nonsensitized rabbits given only the dilute formalin, in sensitized rabbits receiving only the challenging antigen or the irritant, and in nonsensitized animals given both the dilute formalin rectally and the final dose of egg albumin intraperitoneally or intravenously. Examinations of the feces for pathogenic bacteria and parasites in representative groups of animals were negative.

Results

Normal and Control Observation

Normal Rabbit Colon—Gross Observations: The normal rabbit colon mucosa is a grayish-white color, with an almost transparent appearance; vascular markings usually are slight. In rabbits examined several times during the day, the degree of hyperemia may vary, but it is never pronounced. The proctoscopic criteria of colon response were hyperemia, hemorrhage, and ulceration. The degree of hyperemia was graded arbitrarily as +, very slight; ++, definitely pink mucosa; +++, moderate reddening, and ++++, a fiery or cherry-red appearance. In general, the rabbit rectum and colon resisted considerable trauma and manipulation. Repeated or deliberately rough proctoscopy produced hyperemia up to ++; only a +++, and most often a ++++, response was regarded as significant. Hemorrhage and ulceration were not observed in the normal rectum and colon, and their occurrence and severity, together with inflammation, were the principal proctoscopic criteria of a positive response.

Effects of Rectal Irritant (Formalin).—A single "course" of formalin instillations (two rectal instillations of 1 ml. of 1%, occasionally 2%, formalin each, within 24 hours) in normal rabbits usually produced mild to moderate hyperemia, subsiding within several hours. Control observations in 4 rabbits receiving 6 "courses" of dilute formalin (12 rectal instillations) during 12 weeks, in 6 animals given 8 instillations over a period of 4 weeks, and in 2 rabbits, each receiving 1% formalin daily for 5 and 10 consecutive days, indicated varying degrees of, but never severe, hyperemia; scattered tiny hemorrhages and erosions were noted infrequently (Table 1). Only mild hyperemia was observed in the rectum and colon, proctoscopically and at autopsy, in six rabbits given rectal instillations on two successive days every two weeks for a period of six months. In one rabbit given 10 courses of 1 cc. of 1% formalin, and

TABLE 1.—*Effect of Repeated Rectal Instillations of Formalin in Nonsensitized Rabbits*

No. Animals	No. Formalin Instillations	Duration of Observation	Final Rectum-Colon Response	
			Hyperemia *	Hemorrhage, Ulceration
2	5	5 days	2	0
2	10	10 days	2	0
6	8	4 wk.	6	0
4	12	12 wk.	4	0
6	30	6 mo.	6	0

* Varying degree.

TABLE 2.—*Effect of Auer Procedure in Nonsensitized Rabbits*

Challenging Antigen		No. Animals	Rectum-Colon Response
Route	Amt., Mg.		Hemorrhage, Ulceration
I. P.	10	13	0
I. P.	30	18	0
I. V.	10	6	0
I. V.	15	10	0

TABLE 3.—*Effect of Auer Procedure in Rabbits Sensitized to Egg Albumin*

Group	No. Studies	Rectum-Colon Response: Hemorrhage, Ulceration
Antigen only	28	0
Irritant only	48	8*
Antigen I. P. plus irritant	70	36
Antigen I. V. plus irritant	17	15
Total	87	51

* Mild, transient.

five given 1 cc. of 2% formalin, approximately every two weeks during a period of eight months, the rectum and colon grossly appeared moderately hyperemic; minimal inflammation and edema were noted histologically. The changes occurred only in the area of rectum and descending colon exposed to the formalin, as indicated by the pattern of diffusion of similar volumes of methylrosaniline (gentian violet), instilled rectally. The absence of a colon response in the numerous negative tests with small amounts of challenging antigen and in the initial studies with the antigen given rectally constitute, in effect, additional formalin controls. The rectal mucosa was most reactive to the irritant in rabbits restudied after a previous experimental colitis; however, the changes were not pronounced.

Auer Response in Colon

Single ("Acute") Tests.—The Auer procedure caused no obvious changes in nonsensitized rabbits (Table 2). In the sensitized animals the challenging antigen alone evoked no response. Formalin alone produced hyperemia, and, infrequently, a few hemorrhages and erosions. In contrast, rectal bleeding, hemorrhage, moderate to severe inflammation, and ulcerations developed in approximately 50% of sensitized rabbits receiving the challenging antigen intraperitoneally (Table 3). Similar lesions occurred in practically all animals challenged intravenously. The response was evident proctoscopically within 2 to 6 hours after the final injection and appeared to attain a maximum within 12 to 24 hours. The lesions disappeared from proctoscopic view within 24 to 48 hours, occasionally 72 hours. However, repeated injection of antigen alone at intervals of one or two days occasionally renewed the response; in several rabbits an active colitis was maintained thereby for approximately 7 to 14 days.

Recently, sensitized rabbits given the antigen as a retention edema (10 cc. of a

TABLE 4.—*Effect of Auer Procedure on Colon of Sensitized Rabbits—Challenging Antigen Administered Rectally**

	Group	Procedure	No. Animal Studies	Rectum-Colon Response: Moderate	Severe
Auer	Sensitized with PIP	10% egg albumin enema	7	7	0
		10% enema with I. V.	9	9	0
	Sensitized with Freund's adjuvant and multiple portal injections	15% enema	4	4	0
		10% enema	5	5	0
		10% enema with I. V.	9	4	0
		15% enema with I. V.	14	11	0

* Run represents one to four days in succession that antigen is given to animal.

Control studies in nonsensitized rabbits given formalin alone or formalin and the egg-albumin enema indicated rare isolated hemorrhages and hyperemia; sensitized rabbits given formalin or the egg-albumin enema manifested only localized hyperemia or no change.

TABLE 5.—*Effect of Auer Procedure in Rabbits Sensitized with Pentex Albumin* **

Test Animal Group	Procedure	No. Animal Studies	Colon Reaction	
			Moderate	Severe
Sensitized with Freund's adjuvant and multiple portal injections	10% enema with I. V.	8	6	2
Sensitized with multiple portal injections only	10% enema	11	3	3

* Found 93% pure by paper electrophoresis.

10% solution), with the temporary aid of a cotton rectal plug, developed a similar hemorrhagic inflammatory process in the rectum and colon (Table 4). Positive reactions were obtained in 40 of 48 separate studies. The tissue response was characterized by multiple hemorrhages, superficial ulceration, and inflammation. The area of involvement usually extended from the anus to the level of 15 cm., affected diffusely or irregularly; in several rabbits, the severest reaction developed at a level approximately 10 or 12 cm. above the anal orifice. Rectal irritant alone and antigen enema alone in sensitized rabbits produced no changes. The combination of rectal irritant and antigen enema in a nonsensitized animal resulted merely in three tiny submucosal hemorrhages. Oral administration of the challenging egg albumin in the drinking water as single or repeated doses was unsuccessful. At the end of December, 1957, Pentex (five-times-crystallized) egg albumin was substituted for the earlier preparation. This albumin is approximately 93% pure, in comparison with the 52% preparation of albumin administered previously. A more pronounced reaction was anticipated with the purer albumin; this expectation was confirmed (Table 5).

The amount of challenging antigen appeared to be important in eliciting a response. Quantities of 60 mg. intraperitoneally evoked apparently greater responses in a small group of animals than did the dose of 30 mg. The daily intraperitoneal injection of 150 and 100 mg. of antigen for two and four days, respectively, without further rectal irritant after the first day, appeared to prolong the colon reaction for 96 and 120 hours (Table 6). Evidence of "colitis" also was noted in several sensitized rabbits with colitis previously, who several months later received the antigen only. Similar findings were noted in a few sensitized rabbits with previous colitis given the rectal irritant only. The individual responses varied considerably and unpredictably; in some tests the response, as judged clinically and proctoscopically, appeared to be uniformly negative, whereas in other series colon responses were frequent.

Previous studies in our laboratory had indicated that, as in the dog, the injection of methacholine was capable of producing edema and hemorrhage of the rectum and colon in rabbits also. These animals were highly sensitive to the cholinergic drug, and the experiments were difficult to complete because of the high mortality rate. How-

TABLE 6.—*Response in Sensitized Rabbits* * *Given Multiple Injections of Antigen*

Egg Albumin (I. P.)	Group	No. Studies	Rectum-Colon Response		Duration, Hr.
			Negative	Positive	
100 mg. daily — 4 days †	"Auer"	7	2	5	96-120
	controls	4	3	1 (?)	
150 mg. daily — 2 days †	"Auer"	4	1	3	48- 72
	controls	3	3	0	

* Previous Auer procedures.
† After rectal irritant.

TABLE 7.—*Increased Colon Response in Sensitized Rabbits to Repeated Auer Procedures*

Auer Procedure *	Group No. Animals	Rectum-Colon Response: Hyperemia	Rectum-Colon Response: Hemorrhage Ulceration
First	Nonsensitized (6)	0	0
	Sensitized (6)	4	4
Second	Nonsensitized (6)	3	0
	Sensitized (5)	4	5
Third	Nonsensitized (6)	4	0
	Sensitized (5)	5	5 †

* At intervals of two weeks.
† Severe.

TABLE 8.—*Response in Sensitized Rabbits to Multiple Auer Procedures*

No. Auer Procedures	No. Animals	Rectum-Colon Response * Negative	Rectum-Colon Response * Positive
1	6	2	4
5	6	17	13
11	1	9	2

* Duration 6-24 hours (single challenging dose of egg albumin (30 mg. intraperitoneally)).

ever, substitution of methacholine for the formalin in the Auer procedure in a small number of experiments resulted in colon responses with methacholine and antigen, apparently resembling those obtained with formalin and antigen. The observations, if confirmed, might suggest that neurogenic stimulation also may contribute to the "nonspecific" inflammation.

Repeated Auer Tests—"Chronic" Response.—In one group of sensitized animals the colon reaction appeared to increase steadily as the Auer procedure was repeated (Table 7); in nonsensitized rabbits studied concurrently the rectal irritant alone produced a rather constant mild hyperemia and slight hemorrhage. The development of a more chronic colitis was attempted in a second group of sensitized rabbits in which the Auer procedure was repeated approximately every two weeks during a period of approximately six months; 1% formalin was the rectal irritant during the first four months and 2% formalin was the rectal irritant during the last two months. Controls included sensitized and nonsensitized animals given antigen or formalin alone; the challenging dose of egg albumin was 30 mg. intraperitoneally. Arthus tests were repeated prior to each test procedure; rabbits with negative responses were resensitized. As noted previously, the colon reactions were most pronounced in sensitized rabbits challenged with antigen after formalin rectally; however, results were highly variable (Table 8). In one animal examined at elective autopsy, the rectum and lower colon were severely inflamed. Polyp-like growths developed in several rabbits after repeated Auer procedures.

Persistent reactions were noted in the colon for periods up to 144 hours after the final enema in a group of eight rabbits sensitized with Pentex albumin; all had demonstrated positive Arthus reactions. On Day 1, 1 cc. of 1% formalin was instilled rectally. On Days 2, 3 and 4, 1 cc. of 1% formalin was instilled rectally, followed 45 minutes later by 10 cc. of 10% egg albumin rectally. All rabbits were procto-

TABLE 9.—*Persistent Colon Auer Reactions in Rabbits Sensitized with Pentex Egg Albumin*

Rabbit	Type and Duration, (Hr.) of Reactions: 6	12	24	48	72	96	120	144
1	Severe							
2	Severe	Moderate						
3	Severe		Moderate					
4	Moderate			Moderate				
5	Severe				None			
6	Severe					Moderate		
7	Moderate						None	
8	Moderate							Moderate

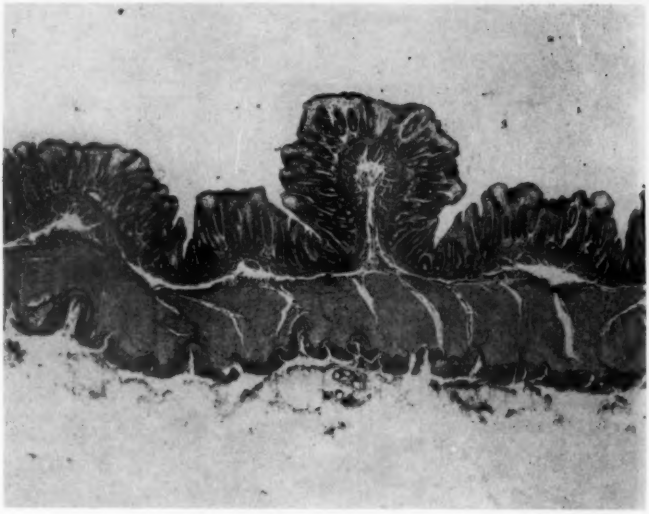

Fig. 1.—Caudal portion of normal rabbit colon. Note double layer of muscularis mucosae and tendency to formation of polyp-like nodule. Reduced to 80% of mag. × 105.

scoped at six hours after the last enema, and one rabbit each was killed at intervals up to 144 hours (Table 9).

The other abdominal organs, examined in representative animals, did not present gross anatomic changes.

Anatomic-Histologic Observations of the Rabbit Colon

Normal Colon.[47,48]—The colon of the rabbit begins at the cecum with a dilated portion, the ampulla caecalis coli. It is approximately one meter long and is divided into several limbs. The first limb has three taeniae, forming three rows of sacculation. Toward the second limb, two taeniae unite and the third joins more proximally, so that only one row of sacculation is present. The caudal end of the colon is thin and resembles small bowel.

Microscopically, the colon of the rabbit in its caudal portion presents crypts of Lieberkühn grouped less closely than in the human colon (Fig. 1). Deep pits separate groups of crypts, 2 to 10 in number. The surface epithelium and that of the pits are cylindrical and take an eosinophilic stain, with the nucleus closer to the surface than to the base of the cell. Goblets cells are not visible. Paneth cells have not been observed. The glands are of simple structure, rising perpendicularly to the axis of the intestine or obliquely when their openings are in the crypts. The glands of Lieberkühn have a cylindrical epithelium, staining more deeply than the superficial epithelium. The lamina propria is sparse and contains only a few cellular elements of mesenchymal nature. Lymph follicles have not been visible in the material studied. The pits contribute to a characteristic appearance of the mucosa, with conically shaped villi, resembling those of the small intestine. The muscularis mucosae is thicker than in man and is composed of two sections; a superficial layer of longitudinal fibers, and a deeper layer of circular fibers. Occasionally, contraction of the muscularis mucosae produces a nodular appearance, simulating polyp formation. The submucosa is very thin. The muscularis propria, occupying approximately half the thickness of the intestinal wall, is composed of longitudinal and circular layers; between them lie the ganglion cells of Auerbach's plexus.

Effect of Formalin.—The microscopic appearance of the colon exposed only to the formalin solution varied considerably. Mild edema and cellular infiltration were noted in normal nonsensitized animals. In sensitized

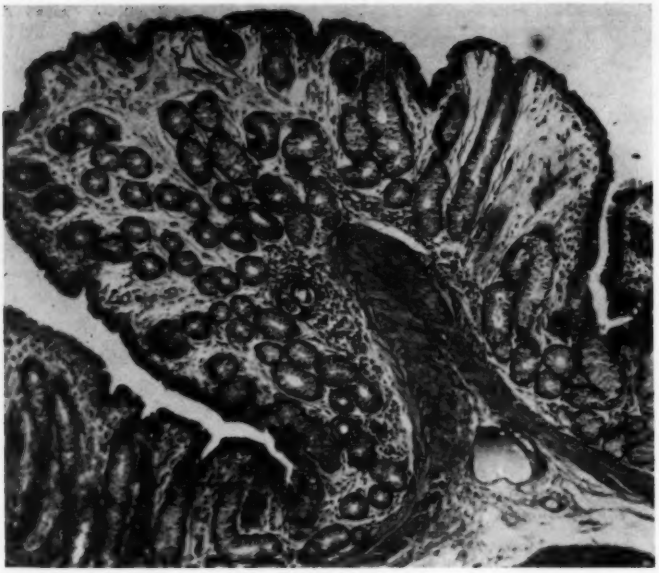

Fig. 2.—Formalin-induced edema in rabbit colon. Reduced to 80% of mag. × 150.

rabbits, formalin alone not infrequently caused focal edema and mild congestion of the mucosa and submucosa, slight submucosal leukocytic infiltration, and occasional small areas of epithelial denudation; hemorrhage was rare (Fig. 2). In sensitized rabbits which had had experimental colitis earlier, the findings consisted of polymorphonuclear-cell infiltration of the mucosa and leukocytic migration through mucosal epithelium, occasional fibrin exudate overlying small areas of damaged epithelium, and, infrequently, hemorrhages; however, the changes were not pronounced.

"Acute Auer Response."—The single Auer procedure in sensitized rabbits pro-

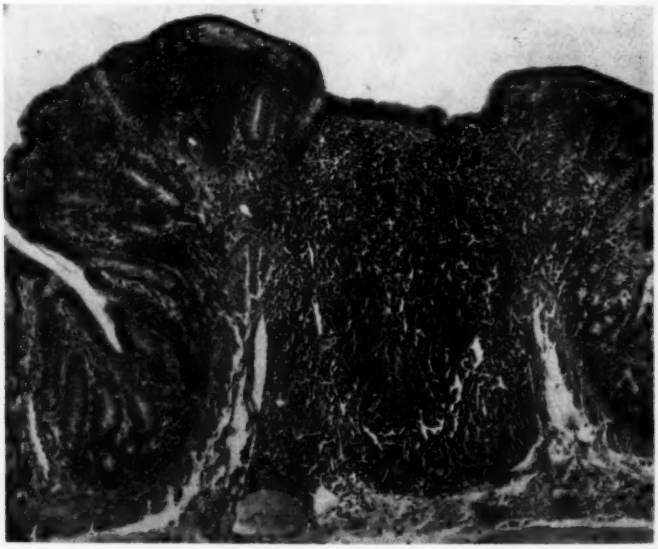

Fig. 3.—Regeneration of colonic epithelium after previous ulceration; large collection of lymphocytes and hemorrhage; after several Auer procedures. Reduced to 80% of mag. × 65.

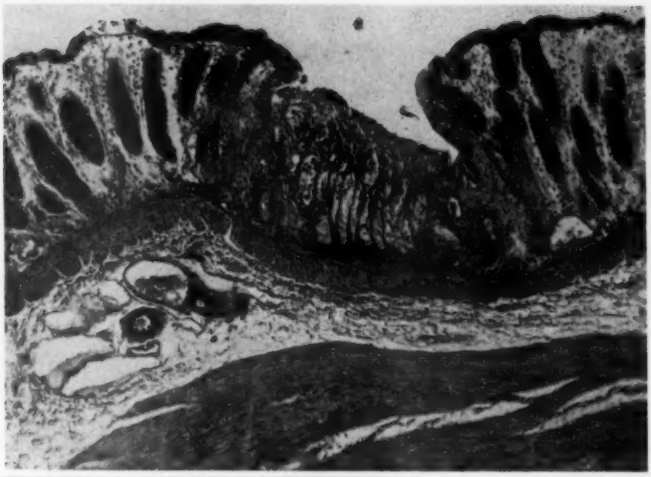

Fig. 4.—Focal necrosis of epithelium with inflammation and edema of mucosa and submucosa in acute "Auer reaction." Reduced to 73% of mag. × 52.

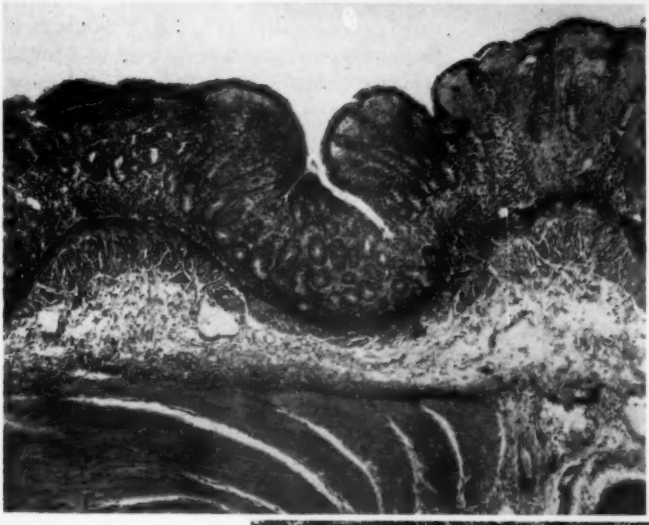

Fig. 5.—Acute "Auer colitis," inflammation and edema, vascular congestion, dilated lymphatics. Reduced to 73% of mag. × 70.

Fig. 6.—Acute "Auer colitis" with ulceration of mucosa. Reduced to 85% of mag. × 80.

duced diffuse edema of the mucosa and focal areas of pronounced edema in the submucosa, hemorrhages originating from subepithelial vascular plexuses and extending partly or completely through the mucosa; frequently cellular infiltration with polymorphonuclear cells, plasma cells, lymphocytes, monocytes, and eosinophils; disappearance of the glandular cells of the crypts; thinning of the muscular layer; dilated lymphatics and blood vessels, and occasional perivascular infiltrates (Figs. 3, 4, 5). Severe necrosis of the mucosa (Fig. 6), occasionally diffuse, but often focal in distribution, developed in several animals. Increased mitotic activity in the deeper layers of the mucosa was observed occasionally. The tissue response after administration of the challenging antigen as an enema was characterized by edema, cellular infiltration with lymphocytes, plasma cells, and eosinophils, focal hemorrhage, ulceration and necrosis (as in the Shwartzman reaction), and perivascular infiltration of small round cells, especially eosinophils. The findings in one rabbit were not unlike those observed in the ulcerative colitis of man. In a few rabbits, intravenous administration of Evans blue (1%) immediately preceding the formalin instilled rectally and the eliciting antigen demonstrated increased capillary endothelial permeability during the Auer reaction.

"Chronic Auer Response."—Acute and chronic inflammation was apparent in sensitized rabbits after several episodes of "colitis." The histologic changes included areas of superficial ulceration and hemorrhage, accompanied by a moderate or severe granulocyte response, and infiltration also with plasma cells and lymphocytes (Fig. 7). The accumulation of lymphocytes frequently extended from below the mucosal epithelium through the muscularis mucosae to the submucosa; some were irregular in outline; others were well organized, with germinal centers. Plasma cells predominated occasionally (Fig. 8). Additional findings were distention of mucous glands, evidence of healed ulceration with distortion of the mucosa, denudation of the glandular epithelium, crypts formed only by thin trabeculae of the lamina propria, and thickening of the muscular layers. The vascular changes included dilated lymphatics, congested veins, thickened arterioles, and occasional perivascular infiltrates, the severity increasing with the intensity of the tissue response.

Pronounced inflammation developed in the rectum and distal colon in one sensitized rabbit given several rectal instillations of

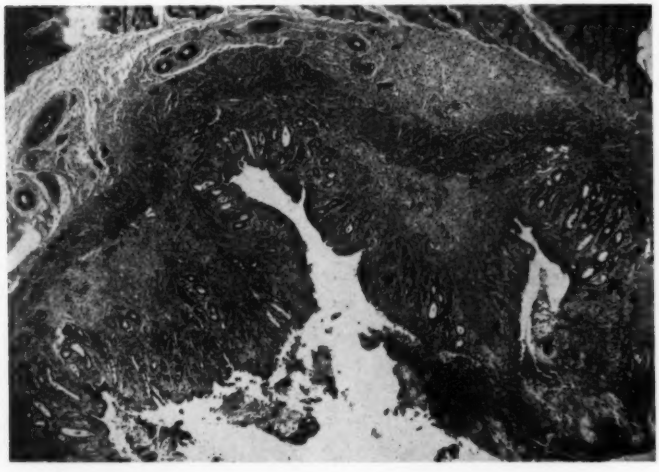

Fig. 7.—"Auer colitis" with ulceration, infiltration with round cells and reticulum cells. Reduced to 85% of mag. × 50.

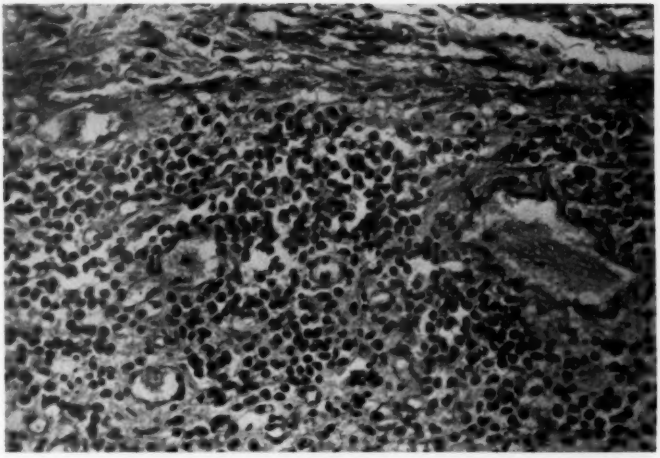

Fig. 8.—"Auer colitis." Pronounced infiltration with lymphocytes and plasma cells. Reduced to 85% of mag. × 380.

2% formalin and two months later sensitized more intensely with the combination of Freund's adjuvant and egg albumin. Approximately one week after a positive Arthus test, 1 cc. of 2% formalin was instilled into the rectum twice in 24 hours, and 45 minutes later followed by 100 mg. of egg albumin intravenously. Proctoscopy several hours later indicated pronounced hyperemia, hemorrhage, and ulceration. Histologic study demonstrated a striking acute and chronic process. The acute changes included diffuse mucosal edema, focal ulceration and epithelial necrosis, severe cellular infiltration, moderate hemorrhage, deposition of fibrin, thrombosed submucosal vessels, and distinct acute perivasculitis. The chronic changes included epithelization of previous ulceration, characterized by depressed areas of simplified epithelium, with absence or atrophy of underlying mucous glands; and glandular distortion; focal hyperplasia of remaining epithelium, and small infiltrates of monocytes. Focal absence of stroma and extension of the mucosal glands through defects in the muscularis mucosae were noted in a few areas.

In animals with previous experimental colitis which were later killed without having received the rectal irritant recently, changes attributable to the past lesions

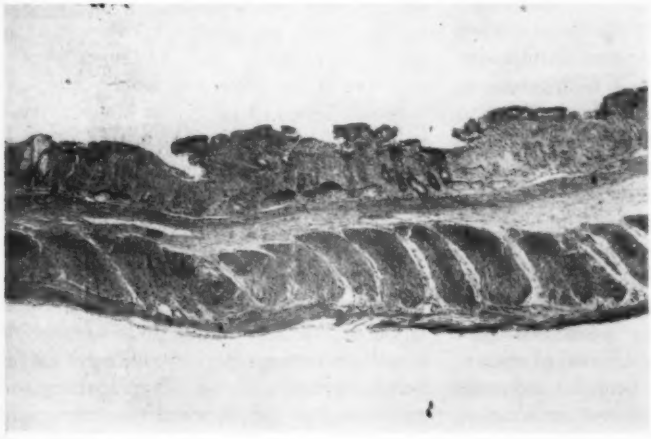

Fig. 9.—Repeated "Auer" reactions in colon. Severely damaged mucosae with pseudopolypoid proliferations. Reduced to 73% of mag. × 35.

Fig. 10.—Repeated "Auer colitis," resulting in atrophy of colon mucosa and thinning of bowel wall. Reduced to 80% of mag. × 52.

could be noted. In several rabbits the healed atrophic mucosa of the colon was not unlike the atrophic colon of healed ulcerative colitis in man. Considerable structural variation was noted in the polyp-like lesions found in some rabbits after multiple episodes of experimental colitis (Fig. 9). A few lesions were organized masses of fibrin with irregular epithelial coverings. In one animal the histologic features included disordered muscularis mucosae, numerous mitotic figures in the epithelium, and cells resembling Paneth cells.

In summary, formalin in nonsensitized rabbits produced mild changes: edema, mild cellular infiltration, slight superficial erosion or ulceration, and infrequent fibrinopurulent exudate. The response to formalin in rabbits with previous experimental colitis was slightly greater. The single Auer procedure in sensitized rabbits produced acute inflammation, cellular infiltration, ulceration, edema of the mucosa and submucosa, disappearance of the glandular cells of the crypts, dilated lymphatics, and perivascular infiltrates. In sensitized rabbits, repeated Auer procedures caused additional pronounced changes: cystic dilatation of crypts; fibrosis of the lamina propria and submucosa; pronounced cellular infiltration, especially with lymphocytes; formation of lymph follicles; epithelial proliferation, hypertrophy of muscle, and questionable papilloma formation. A thinned, atrophic mucosa, not unlike that observed in human ulcerative colitis, was observed after a previous experimental colitis (Fig. 10).

Comment

The present study is of interest in demonstrating the responsiveness of the colon of the rabbit to hypersensitivity reactions.[49] Both the Arthus test and the Shwartzman phenomenon, an immunologically nonspecific process not requiring antigen-antibody union, could be produced readily in the colon of rabbits. The development of a reaction in the colon resembling the Auer response seems of particular interest. Auer, in 1920, had suggested that this phenomenon might be reproduced in any tissue capable of an anaphylactic reaction, including the gastrointestinal tract. Theoretically, the increased capillary permeability and edema, accompanying the formalin irritation, may have facilitated the local accumulation of injected antigen in an area of the bowel containing specific antibody, as has been demonstrated by other investigators under similar experimental conditions; and

may have precipitated thereby a local antigen-antibody reaction. The exact mechanism of fixation of antigen in irritated tissues is not known. The toxic nature of the antigen-antibody reaction in the colon, as with similar processes elsewhere in the body, also is not well understood. This problem currently is being studied.[50] In sensitized rabbits with previous "colitis," injection of the antigen alone occasionally renewed the colon response, in the absence of additional rectal irritant. The duration of this effect varied, and perhaps was limited by antibody exhaustion. In one group of rabbits repetition of the Auer procedure appeared to increase the responsiveness of the bowel, possibly reflecting larger accumulations of antigen and antibody in the rectum and colon; however, in another group this trend was not observed. In some rabbits, colon reactions persisted for as long as six days after the Auer procedure. The experimental situation, of course, differs considerably from a potential clinical state, in which the antigen (e. g., bacterial products, altered tissue protein) might be present more or less constantly; such conditions probably would be more likely to facilitate a sustained reaction. The lack of a response in sensitized rabbits given the challenging antigen orally is not surprising, for administration of egg albumin by this route does not insure passage to the appropriate area in the colon, in an unaltered and effective state. The pronounced colon response in rabbits given the egg albumin as a retention enema would suggest the passage of specific antigen through damaged mucosa, and perhaps greater concentration of the antigen locally; the chronic round-cell infiltration and the perivascular infiltrates containing eosinophils in these animals are of interest. Though some of the histologic changes may resemble ulcerative colitis,[51] the experimental colitis and the human disease are distinguished readily.

The frequency and the intensity of the "Auer response" in the colon varied unpredictably; at times no reaction was evident proctoscopically or anatomically. However, fluctuating results are not unusual in immunological experiments. The differences may be related in part to variations in degree of sensitization, in the contact of the irritant or the eliciting antigen with the mucosa, and in the amounts of antibody and antigen accumulating in the colon. Although the experimental conditions are compatible with an antigen-antibody reaction as the basis of the "colitis," this relationship as yet cannot be regarded as established. Objective evidence of antigen and antibody localization is necessary—perhaps with fluorescein- or isotope-labeled antigen and/or antibody, or other techniques for demonstrating antigen-antibody reactions; such studies now are in progress in our laboratories. The circumstantial evidence, nevertheless, seems attractive.

The experimental findings, though not necessarily indicating an immunological basis for ulcerative colitis, heighten interest in this possibility, at least in some cases. The presence of immunological phenomena in ulcerative colitis and their potential effects have not been investigated adequately. The new immunological techniques now available should help to clarify this important question. The observations seem to be of most immediate interest in suggesting a procedure for inducing colitis experimentally. Many additional problems require investigation. Some of these concern increased reproducibility of the acute Auer response and methods for inducing more intense and more chronic reactions. The action of irritants biologically more appropriate than formalin—perhaps dilute Staphylococcus toxin, bacterial endotoxins, other bacterial products, or humoral, cholinergic, and pharmacological agents—should be studied. An important series of problems relates to the fundamental nature of the tissue reaction: more definite evidence of antigen-antibody union during the Auer response in the colon; correlation of the reaction with serum-antibody titers to egg albumin; possible alterations in the pattern of the serum proteins, with particular ref-

erence to γ-globulin; further study of oral and rectal routes of administration of the challenging antigen, and use of other antigens and other animal species. Detailed anatomic studies of the evolution of the Arthus and Shwartzman reactions in the colon of rabbits are described elsewhere.[27-29,32] The effect of these processes combined with the Auer procedure upon the colon also may be of interest. Finally, the ultimate course and anatomic features of prolonged experimental colitis would appear to be important in relation to the possible development of atrophy, epithelial hyperplasia, and polyp-like structures. This experimental procedure thus may facilitate numerous studies of the response of the colon of animals to immunological damage; the information gained thereby may contribute to a better understanding of the reaction of the colon to injury in general, and perhaps to the pathogenesis of ulcerative colitis.

Conclusions

1. A "colitis" may be induced in rabbits sensitized to egg albumin, following mild rectal irritation and then challenging with egg albumin systemically or rectally.

2. The colitis presumably develops on the basis of the Auer principle: sensitization, generalized antibody response, localization of the antibody and the challenging antigen in the rectum and colon, and combination of the antigen and antibody in the wall of the colon.

We are especially indebted to Dr. Robert W. Wissler, Professor of Pathology, The University of Chicago School of Medicine, who constantly provided guidance and many helpful suggestions during the course of the study and who helped evaluate the histologic material. We also wish to acknowledge the valuable technical assistance of Dr. Willard Smith and Mrs. Jean Schoolcraft during the earlier phases of the study.

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A Simplified Rabbit Ear Chamber

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The rabbit ear chamber technique elaborated by Sandison [1] and later modified by Clark [2] offers a unique method for studying the circulation of living tissue. It affords a method for studying the course of infections,[3,4] the tissue response in hypersensitivity states,[5] the anti-inflammatory effects of corticoids,[6] and the growth of tumor cells.[7]

The techniques heretofore described for making and for implanting the ear window are complicated, and the incidence of infection and of slough of the chamber is high. The chambers described by Sanders [8] and by Ahern and associates [9] require a skilled machinist and the facilities of a well-equipped shop. Placement of these chambers necessitates punching four holes through the rabbit's ear and denuding the cartilage of skin and, to some extent, of blood vessels over a comparatively large area on both sides of the ear. When placed in the ear, the chamber is open at its circumference, and bacterial infection or epithelium may gain access to the observation table. A major drawback to Ahern's chamber is the inaccessibility of the tissue. The cover slip cannot be easily removed and replaced, and the central, removable plug creates a "blind spot" in the center of the observation area.

The chamber described here overcomes most of the above difficulties. It can be easily assembled in any laboratory at a small cost and with a few simple tools. Placement of the chamber in the ear requires the punching of only one hole and the stripping of the skin off cartilage on only one side of the ear. The observation area is sealed off from the outside skin, and the cover slip can be easily removed and replaced.

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Materials

The following materials are required: a sheet of polyvinyl, $\frac{1}{32}$ in. thick; 4-mil-thick Teflon wettable on one or both sides; 1-mil-thick Teflon wettable on one side; circular mica coverglasses $\frac{1}{2}$ in. in diameter; Lucite rod $\frac{3}{4}$ in. in diameter; Lucite rod $\frac{5}{16}$ in. in diameter; $\frac{1}{2}$ in. stainless steel expansion rings, and bottle of ethyl acetate. The tools needed in the laboratory are a set of cork borers; a set of hand punches, including a $\frac{3}{4}$ in. and a $\frac{1}{2}$ in. punch; a hammer, and a $\frac{1}{16}$ in. punch.

Drill a hole $\frac{1}{2}$ in. in diameter down the center of the $\frac{3}{4}$-in. Lucite rod and cut it

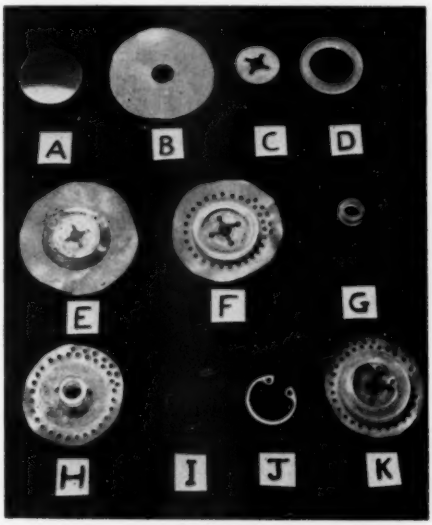


Figure 1

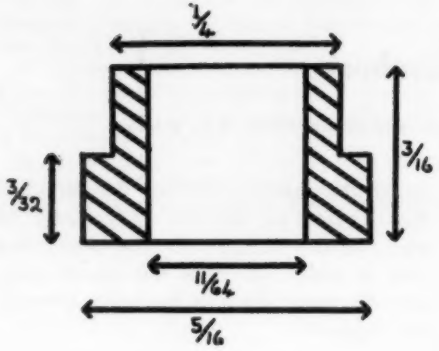


Fig. 2.—Lucite peg.

into rings, approximately 1/16 in. high, with an outside diameter of 3/4 in. and an inside diameter of 1/2 in. (Fig. 1*D*). Drill a 11/64 in. hole down the center of the 5/16-in. Lucite rod and cut off into 3/16 in. lengths, after first turning a 3/32 in. shaft, 1/4 in. in diameter on one end, to produce a peg, as pictured in Figure 1*G*. A cross section through the center of this peg is shown in Figure 2. These Lucite rings and pegs are the only machined parts and can be turned out in quantity very cheaply.

Assembly Procedure

Using a 3/4 in. punch and a hammer, punch out circles from the polyvinyl sheet (Fig. 1*A*). By placing a second sheet of polyvinyl under the one being punched, shattering is prevented. Cut out 1 1/2 in. circles of 4 mil Teflon and cut a hole 1/4 in. in diameter in the center with a cork borer (Fig. 1*B*). Center the polyvinyl disk over the hole in the Teflon on its wettable surface and bond the two together. A drop of ethyl acetate placed at the edge of the polyvinyl will run by capillary action between the two parts without marring the central area of the disk, and firm pressure for a few seconds will secure a tight union.

Using a 1/2 in. punch, cut circles from the 1 mil Teflon, then cut out a central hole with a No. 2 cork borer to produce a Teflon washer. With a pointed scissors cut four channels, 1/16 in. wide, in the washer to produce a "spacer" (Fig. 1*C*). This spacer will separate the coverglass from the observation table and determine the thickness of the vascularized tissue. Center the spacer over the polyvinyl disk so that its hole is concentric with the hole in the Teflon on the other side of the disk and bond the spacer in place with ethyl acetate (Fig. 1*E*). Now punch four holes 1/16 in. in diameter through the polyvinyl disk, placing them at the ends of the channels in the spacer. These holes should fall just outside the central hole in the 4 mil Teflon. Bond the Lucite ring to the rim of polyvinyl surrounding the spacer. Perforate the 4 mil Teflon with a circle of holes placed about 1/8 in. away from the edge of the polyvinyl disk (Fig. 1*F*). This can be done by using a No. 18 gauge needle with its point ground off squarely and the rim honed to a cutting edge. With scissors cut the Teflon to a circular collar leaving a 1/8 in. rim outside the holes. Now bind the Lucite peg onto the back of the chamber, to the polyvinyl exposed by the central hole in the Teflon collar (Fig. 1*H*). Clean the chamber by dipping it several times in a hot detergent, rinse thoroughly with tap water, and dry. Drop the coverglass (Fig. 1*I*) into position, inside the Lucite ring, and secure it in place with an expansion ring (Fig. 1*J*). Sterilize the chamber (Fig. 1*K*) under an ultraviolet light. The depth of the chamber can be measured with the scale on the fine focusing knob of a microscope by focusing up and down between the table surface and the coverglass surface and should measure about 70μ.

Placement of the Chamber

Use rabbits with ears 6 in. long. Clip the hair off the ears, and wash them well with pHisoDerm the day prior to surgery. Anesthetize the animal with intraperitoneal pentobarbital (Nembutal) (60 mg/kg. body weight), and again wash the ears with

pHisoDerm.* Punch a hole with a No. 3 cork borer 1¾ in. from the tip of the ear and immediately adjacent to the lateral edge of the central artery. Free the skin with its underlying perichondrium from the cartilage on the inside of the ear for a distance of ½ in. about the hole. This is the most critical part of the whole procedure and will determine the success or failure of the preparation. A dissector for this purpose can be made by grinding the point off the blade of a dissecting scalpel to form a semicircular end with a dull edge. The skin must not be split to leave islands of epithelium behind on the cartilage, nor must the dissection be deep enough to tear a hole in the cartilage. On the skin just freed and centered about the hole, make a circular mark with a No. 9 cork borer to guide the trimming away of enough skin to expose a circle of cartilage ¾ in. in diameter. Fill the chamber with saline through one of the four holes with a needle on a syringe. Manipulate the Teflon collar of the chamber under the remaining loosened skin flap; then ease the Lucite peg through the hole in the cartilage. The skin should lie on top of the Teflon collar and fit snugly about the Lucite ring. The under side of the chamber and its four holes lie on top of the cartilage. For the first week, carefully clean away any dried blood about the chamber and cover the skin with a light coating of Polysporin ointment.† The animal should also receive a daily injection of Combiotic.‡

Blood vessels grow through the four holes surrounding the peg and along the channels cut in the spacer. They appear on the central observation area in about 14 days and completely vascularize it in 4 weeks. The tissue is fully mature and suitable for experiments within six weeks.

The appearance of the chamber in the rabbit ear from its front and back surfaces is shown in Figures 3 and 4, respectively.

* pHisoDerm, a synthetic emollient detergent, containing petrolatum, hydrous wool fat cholesterols, sodium octylphenoxyethoxyethyl ether sulfonate, and water, manufactured by Winthrop Laboratories, New York.

† Polysporin, polymyxin B-bacitracin ointment, manufactured by Burroughs Wellcome & Company, Inc., Tuckahoe, N. Y.

‡ Combiotic, containing sodium penicillin G, U. S. P., potassium penicillin G, U. S. P., and dihydrosteptomycin, manufactured by Charles Pfizer & Company, Inc. The Combiotic was kindly supplied to us by the Chas. Pfizer & Company, Inc., Brooklyn, N. Y.

Fig. 3.—Front of chamber in situ.

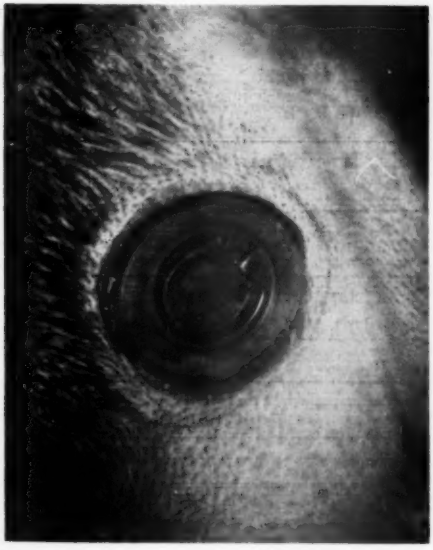
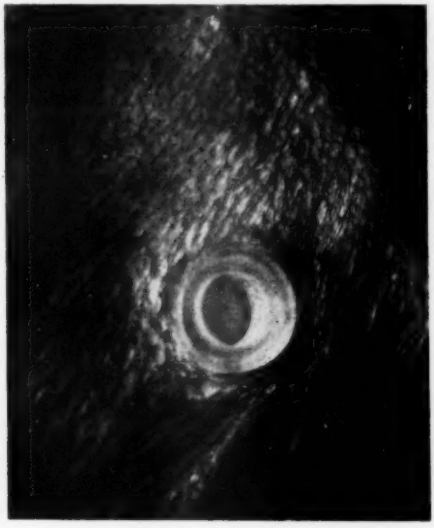


Fig. 4.—Back of chamber in situ.



A Lucite stage plate with a hole that accommodates the peg on the back of the chamber should be made and attached to the mechanical stage of the microscope. Four slide clips may be used to hold the chamber down on the Lucite stage plate.

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Histoplasmosis

In Vivo Studies in the Rabbit Ear Chamber

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The clinical course of histoplasmosis and the pathology of the disease, as seen in the stained tissue sections, have been extensively described. Study of early, experimental histoplasmosis has shown that the spore wall breaks down in the tissue, liberating parasitic yeast cells, which are subsequently disseminated via the blood stream throughout the body.[1] This paper presents the course of Histoplasma capsulatum infection in living tissue and the difference in the tissue reactions to primary and to secondary histoplasmosis.

Materials and Methods

Since the development of the rabbit ear chamber by Sandison[2] many modifications in the construction of the chamber have been devised. The latest modification, by Barclay and associates,[3] is a chamber with a removable cover slip that exposes a layer of vascularized connective tissue 50μ to 75μ thick. The infectious material to be studied can be placed directly on the surface of this vascularized tissue and covered with a new cover slip. This modified chamber was utilized in all the experiments here described.

H. capsulatum spores were obtained by scraping a mycelial growth grown on corn meal extract agar plates and suspending the mature tuberculate chlamydospores in saline. The suspension was centrifuged at 2,500 rpm for 15 minutes, and the supernatant fluid was aspirated, leaving a heavy deposit of live spores. A blunt platinum wire was dipped into the spores and then touched once to the vascularized connective tissue of the chamber. The number of spores deposited in each experiment could easily be counted, and the degree of infection was approximately the same through the experiments described. Ten primary infections were studied in detail. Of these 10 rabbits, 5 became skin-test positive to 1:10 histoplasmin, and the chambers in the contralateral ears were infected and the reinfection process compared with the course of the primary infection. Thus, each animal served as its own control.

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Department of Medicine, University of Chicago, The School of Medicine.

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Seymour Coman Fellow, the University of Chicago, Department of Medicine (Dr. Farid). Present address: Naval American Medical Research Unit #3, Cairo, Egypt, U. A. R.

Results

The uninfected, mature chamber consisted of a clear, connective tissue stroma, which supported venules, arterioles, capillaries, and lymphatics. No free fluid was present in the connective tissue, but scattered histiocytes could be seen throughout the tissue, and a few polymorphonuclear leukocytes could be observed. Blood flow in the vessels was usually rapid, the red blood cells moving in the center of the stream, while occasionally leukocytes moved at a slower pace in the periphery of the blood flow. The vascular endothelium was well defined.

Primary Infection.—Removal of the cover slip and inoculation of the H. capsulatum spores caused minimal damage. A few free red blood cells became scattered in the extravascular spaces, and blood flow in the vessels became slow for approximately 30 minutes. Within one hour of infecting the tissue with spores, leukocytes could be seen adhering to the vascular endothelium. This collection of leukocytes on the endothelium increased until, in many areas, they seemed to form a continuous cellular lining on the inner surface of the vessels. Between two and three hours after infection white blood cells passed out of

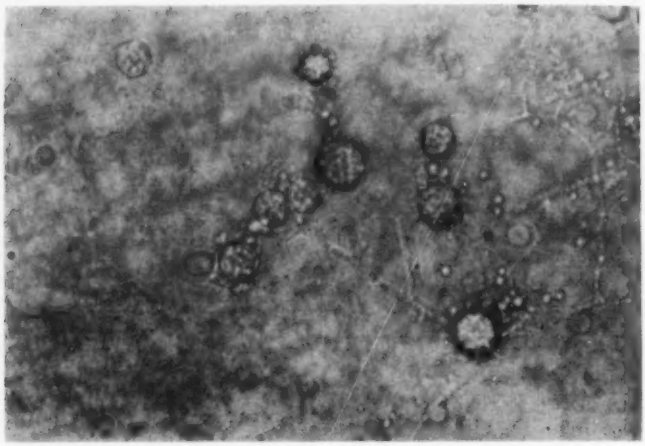

Figure 1

the vessels into the tissue spaces and moved toward the spores (Fig. 1). During the fourth hour leukocytes surrounded the spores, each spore being walled off by three to eight leukocytes (Fig. 2). Although some positive attractive force seemed to exist between the spores and the leukocytes, as evidenced by selective grouping of the leukocytes about the spores and mycelial elements, many of the leukocytes were seen to come up to and touch a spore, and then move away as if repelled. This initial tissue reaction was identical in both primary and reinfection histoplasmosis and occurred at the same rate under both circumstances. During the first two days of infection, there continued to be an outpouring of leukocytes and fluid into the tissue spaces. By the fourth day the spores were surrounded by a mass of closely packed leukocytes. By the sixth day a few spores were seen inside large macrophages (Fig. 3), but the majority of the spores were surrounded by epithelioid-like cells, without actually being engulfed by them. At this time considerable clearing of exudate and cells occurred. The thickness of the tissue diminished, and blood flow returned almost to normal. There was very little white-cell sticking, minimal vascular dilatation, and only a moderate increase in the rate of blood flow. During the second week of infection the

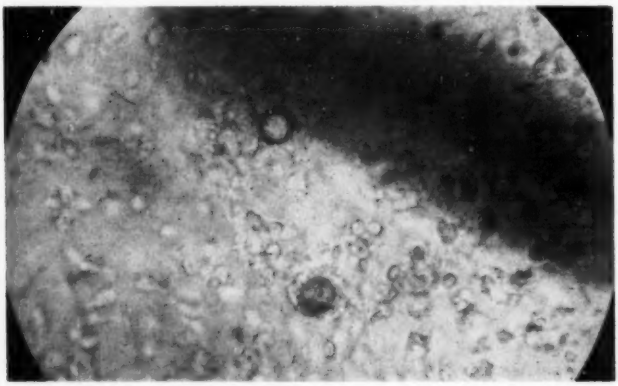

Figure 2

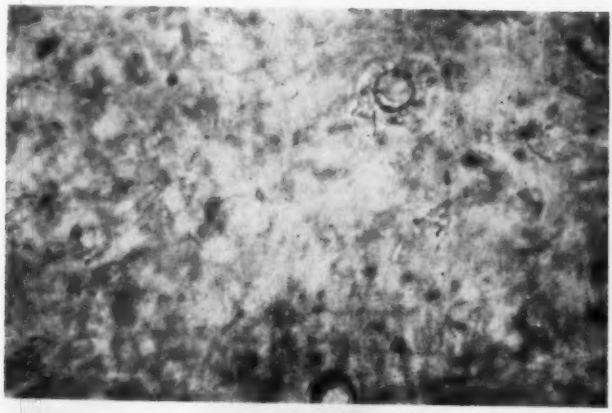

Figure 3

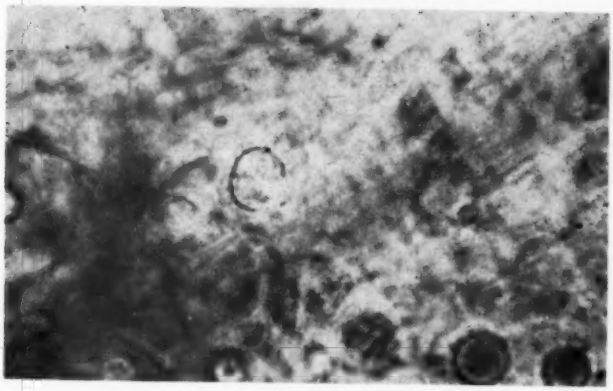

Figure 4

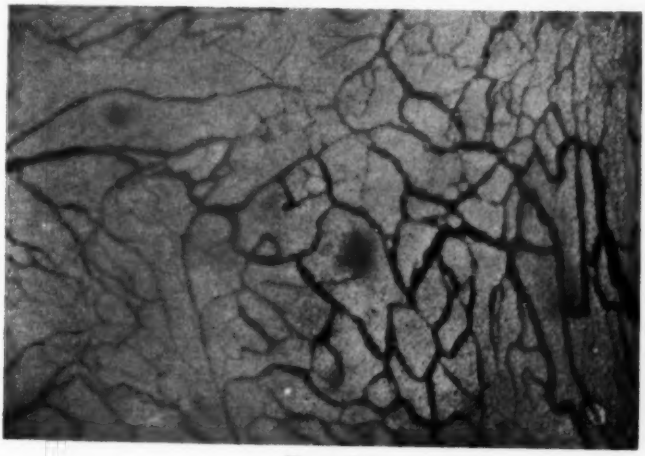

Figure 5

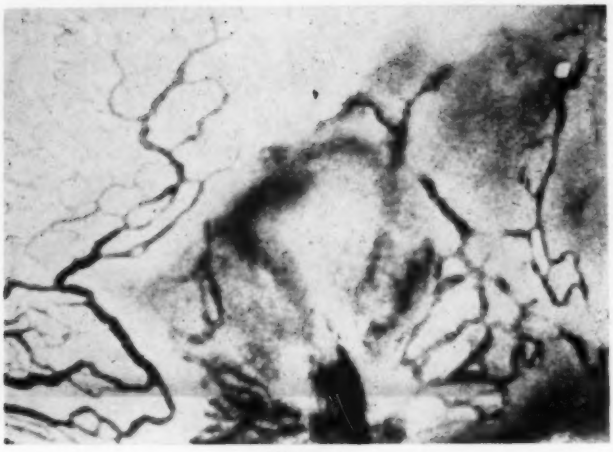

Figure 6

spore contents shrank or became segmented, and this was followed by disintegration of the spore walls. By the ninth day many of the spores could be seen with fractured walls and devoid of any internal contents (Fig. 4). A second inflammatory reaction started in the tissue concomitantly with the breakage of the spore walls and the liberation of yeast cells into the tissues. This late inflammatory reaction was progressive and was characterized by vascular dilatation, hemoconcentration, and massive exudation of fluid and cells (Fig. 5). Finally, the vessels in the immediate area of the spores thrombosed, and this thrombosis gradually extended peripherally from the site of infection (Fig. 6). By the end of the third week necrosis and abscess formation had involved the whole chamber. Chambers destroyed in this manner showed no tendency to revascularize over the six-month period during which they were observed.

Secondary Infection.—The immediate tissue response to H. capsulatum spores was identical in the sensitized animal and in the animal with primary infection. Moreover, the changes in the spores themselves and the breakdown in the spore walls paralleled primary infection. The major difference lay in the vascular response. The initial inflammatory response, occurring

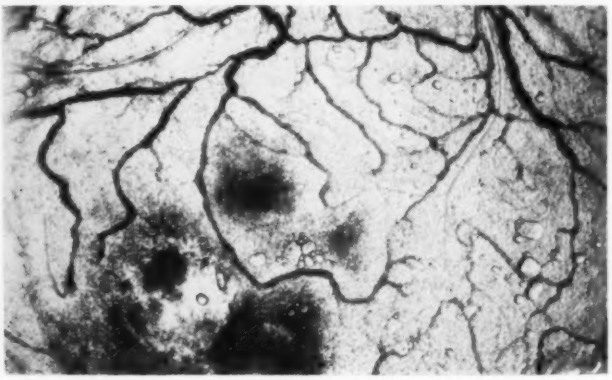

Figure 7

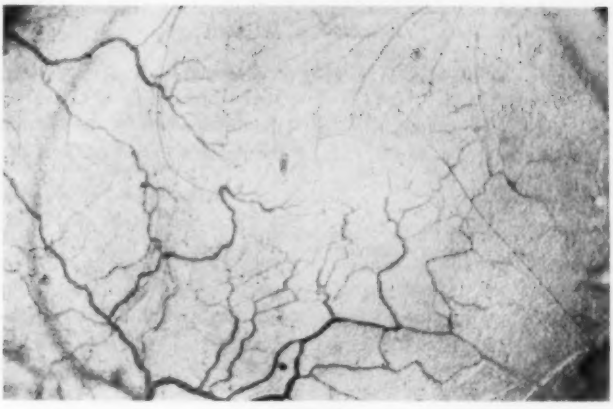

Figure 8

within two to four hours of infection, did not subside, but continued to grow in intensity and extent during the first week of the infection. By the fifth day a central, avascular area formed at the site of inoculation. This area was densely packed with inflammatory cells and was surrounded by grossly dilated, tortuous blood vessels. At the edge of the developing abscess, the venules underwent thrombosis, and in some cases red blood cells extravasated into the tissue spaces. Between the seventh and the ninth day a well-formed abscess was surrounded by a zone of greatly dilated vessels (Fig. 7), this, in turn, being surrounded by relatively normal-looking vascular tissue at the chamber edge. From the 10th day onward the inflammatory reaction gradually subsided. Capillary loops extended into the central core of the abscess, and gradually the whole area became revascularized. Debris was carried away, and the chamber returned to its preinfection appearance (Fig. 8). However, under high-power magnification the intervascular spaces were more granular and cellular, without the fibrous-connective-tissue appearance characteristic of a noninfected chamber. Six months after healing of the secondary infection there was no reactivation of the infection.

Comment

These experiments demonstrated the preliminary changes in H. capsulatum spores in living tissue. A small proportion of the spores were completely engulfed by macrophages before they broke up; the remainder of the spores were surrounded by polymorphonuclear leukocytes and epithelioid cells. The spore wall eventually disintegrated to liberate the parasitic yeast phase of the organism. With the liberation of these cells, and their dissemination throughout the body, there developed a positive skin reaction to histoplasmin and an intense local inflammatory reaction at the site of infection. The primary infection was progressive, and immunity developed too late to limit extensive tissue destruction at the inoculation site. In reinfection histoplasmosis the inflammatory response was immediate and intense, with localized tissue destruction but with restriction of the spread of the infection. The healing process began early, before the end of the second week, and proceeded to complete resolution of the infected area. In these experiments, once resolution of reinfection histoplasmosis had occurred, there was no tendency toward recrudescence of the infection, even though spores could be seen in the revascularized area for as long as one month after their implantation.

The H. capsulatum spores were obtained with the kind cooperation of Dr. John J. Procknow, Department of Medicine, The University of Chicago, who also gave valuable advice in the course of these experiments.

Department of Medicine, The University of Chicago, 950 E. 59th St. (37) (Dr. Barclay).

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Disseminated Sarcoidosis with a Marked Granulomatous Arteritis

ELIZABETH BOTTCHER, M.D., Rochester, N. Y.

Sarcoidosis is a granulomatous disease of unproved etiology. Since the first histologic description by Boeck, in 1899, numerous studies have been made on this disseminated disease. Among the theories of its etiology are that it is an infectious granuloma, a chemical intoxication, a disorder of lipid metabolism, and an exceptional hyperimmune tissue response.[1-3]

The sarcoid granuloma is a noncaseating tuberculoid granuloma made up of radiating histiocytes and varying numbers of lymphocytes and giant cells. Sarcoidosis is a disseminated disease in which the sarcoid granuloma is the characteristic histologic feature. However, sarcoid granulomas may also occur as a local inflammatory response to a myriad of agents, ranging from sand to carcinoma cells.[4] It is also one of the characteristic reactions found in allergic diseases.[5-7] That the same basic granulomatous lesion is found in such diverse conditions is confusing. A common denominator behind so many seemingly unrelated conditions has not as yet been found.

The purpose of this report is to present the pathologic findings in a patient with an allergic history who developed a disseminated sarcoidosis with a marked vascular component. The distribution of the lesions is similar to that seen in Wegener's granulomatosis [8,9] or allergic angiitis but lacks the necrotizing component characteristic of Wegener's granulomatosis. It is a chronic granulomatous arteritis in this case. The ease with which the elastic tissue of the arteries is dissolved here is remarkable. This is in contrast to the difficulty in dissolving elastic tissue experimentally by any means other than enzymatic and also to the difficulty in making it antigenic. The possibility that elastic tissue may act as an autoantigen and cause vascular disease is being investigated experimentally in this laboratory at the present time. In sarcoidosis it is most unusual to have such alteration in the elastic tissue.[10,11]

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Report of a Case

History

This 55-year-old woman had first come to the hospital eight years previously with migratory joint pain, tiredness, and low-grade fever, of three weeks' duration. This was diagnosed to be acute rheumatoid arthritis. She was treated with penicillin because a mucopurulent discharge with a few streptococci was found in her posterior pharynx. Her blood pressure was normal. She was discharged improved.

Two years later she returned with a complaint of ringing in her ears. Her blood pressure was now 190/110 but was lowered with amobarbital (Amytal) sodium. About this same time she began having intermittent asthmatic attacks, described as following colds. Occasional attacks of arthritis continued. These were treated at home with oxytetracycline (Terramycin), after which she developed stomatitis.

Abdominal pain became severe and kept bringing her to the surgeons for multiple operations. The first was a dilation and curettage, followed later by a hysterectomy. Myomas and adenomyosis of the uterus were found, but, in addition, focal areas of inflammation of the large and small bowel were seen at operation. Because of this inflammatory reaction she was again put on antibiotic therapy. She improved and went home, but returned after one month with abdominal pain. This pain gradually subsided without further treatment. The following year she was admitted for a hemorrhoidectomy and later for treatment of a severe asthmatic attack.

Renal dysfunction was first noted two years before her final admission. At this time she entered with weakness, fatigue, weight loss, and polyuria. The blood urea nitrogen was 37 mg. %,

NPN 53 mg. %, and creatinine 2.8 mg. %. A test for uroporphyrins was negative. Eosinophils were now 11%. It is of interest to note that on each admission the eosinophil count was higher, starting at 1% on the first admission and reaching a high of 11%. X-rays, which before had shown only emphysema, now showed diffuse linear fibrotic areas in both lung fields.

The next year a cholecystectomy was performed because of a gallbladder type of pain in her right subscapular area. Cholelithiasis was found. The NPN was still elevated; the serum proteins measured 6.8 gm. %, albumin 3.9 gm. %, and globulin 2.9 gm. %.

Postoperatively she continued to have pain, but went home. Since the pain did not subside even after several months and the cholangiogram showed delayed emptying, the sphincter was dilated and a T-tube inserted. The NPN was now 127 mg. %, and the urine had a specific gravity of 1.009. However, she did fairly well postoperatively, until she developed an acute asthmatic attack. She died rather suddenly the next day.

Necropsy

The stomach was acutely dilated. The heart was of normal size, although the left atrium was thicker and more fibrous than usual and the myocardium was brown. The lungs were less crepitant than usual and had focal dense areas, which on section were firm and fibrous but not well circumscribed. A few bronchi and bronchioles contained mucous plugs. The kidneys were small, together weighing only 160 gm., and were pale and granular with small granular calculi in some of the calyces. In the pancreas there were focal areas of fat necrosis. The gastrointestinal tract, except for the dilated stomach and recent duodenal incision, was normal. The brain was normal except for slight frontal atrophy. The vessels were soft. The aorta was normally elastic with little arteriosclerosis.

Microscopic Study

A severe, sarcoid-like, granulomatous reaction was seen in the lungs, heart, kidneys, mesenteric blood vessels, and periadrenal vessels and ganglia. Surprisingly, none was found in the spleen, and only rare granulomas were seen in the portal areas of the liver.

The granulomas were made up of irregularly arranged mononuclear cells "epithelioid cells," giant cells, and a few lymphocytes. No necrosis was seen. The amount of fibrous tissue varied from none, in early lesions, to almost entirely dense hyalinized tissue, in older lesions.

The granulomas tended to be related to the wall of blood vessels, especially arteries, but also of veins. In some vessels they were in the adventitia; in others, the media. Even the endocardium was not spared (Fig. 1). The elastica was completely dissolved in these areas. Figure 2 illustrates the remarkable moth-eaten appearance of a pulmonary artery. It is also worthy of

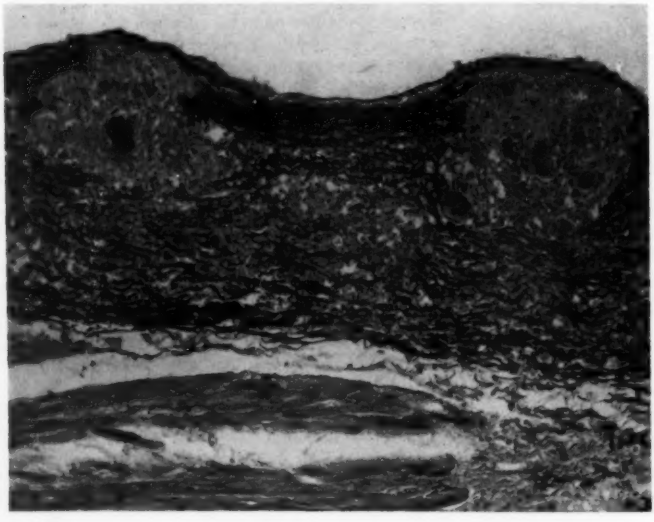

Fig. 1.—Sarcoid granulomas with local destruction of elastic tissue in endocardium. Verhoeff's elastin stain.

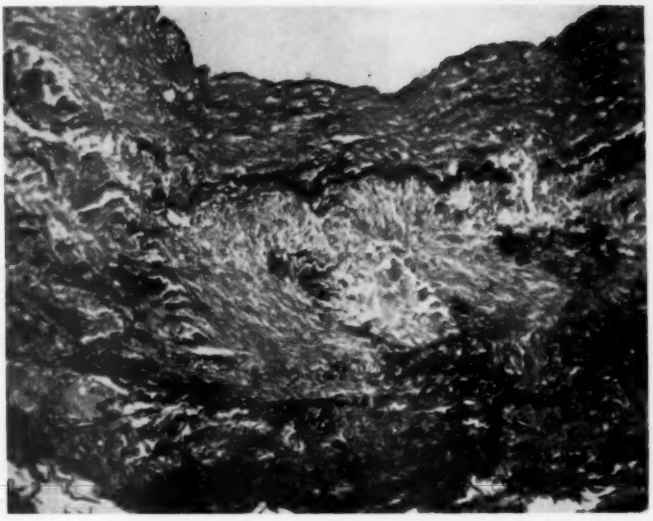

Fig. 2.—Pulmonary artery with marked scarring and loss of elastic tissue. Verhoeff's elastin stain.

note that the arteriosclerotic intimal thickening was more marked over the damaged areas. No rupture of any vessel was found. It was no doubt fortunate for the integrity of the vascular channels that the granulomata were accompanied by sclerosis.

The giant cells contained several types of inclusions. Nearly every giant cell contained small vacuoles with a dot in the center. These gave the appearance of accumulating in clumps and then of suddenly being obscured by the radiating projections of an asteroid body (Fig. 3). Some giant cells had more than one such asteroid body. These asteroid inclusions stained deeply with orcein, while the vacuoles did not. Neither took up the fat stain, oil red O, in frozen sections.

Small light-yellow granules were numerous in macrophages and giant cells, both

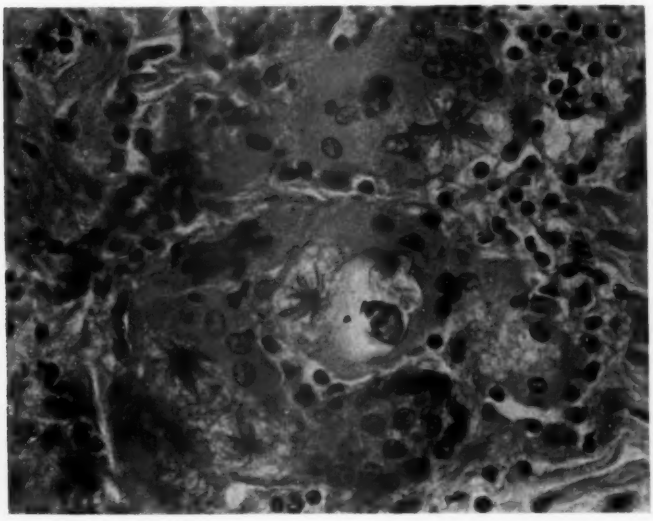

Fig. 3.—Giant cells containing asteroid bodies, anisotropic inclusions, and vacuoles. Hematoxylin and eosin stain.

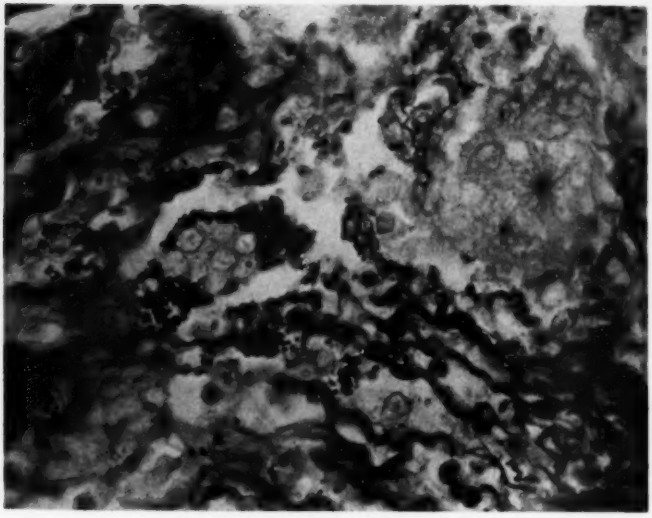

Fig. 4.—Granules within small giant cells and macrophages. Methenamine silver stain.

within and adjacent to the granulomas. They were especially prominent in the younger, less sclerotic lesions, where elastic tissue was being dissolved (Fig. 4). These granules are periodic-acid-Schiff-positive and stain with methenamine silver and Giemsa stains. Their significance is unknown.

Anisotropic crystals (Fig. 3) were seen within the giant cells. They were especially numerous in both heart and lung. Qualitative spectrographic analysis * of incinerated lung and kidney tissue revealed that no unusual element, such as beryllium, was present. Slightly more silicon was found than in the control tissue, but much less than would be found in silicosis. Calcium was the only element that was present in a much greater amount than in the controls. Since Schaumann bodies, concentrically laminated concretions containing both iron and calcium, were numerous, this finding was not surprising. In the kidney these bodies attain a large size.

In addition to sarcoid-like granulomas, numerous Schaumann bodies, and anisotropic crystals in the kidney, there was also a severe glomerulosclerosis. Many glomeruli were completely hyalinized; other areas were completely aglomerular, and other glomeruli contained a few neutrophils. Both arteries and arterioles were extremely sclerotic and thick-walled. In the pelvis a few of the vessels were surrounded by giant cells, but in the parenchyma the vascular lesion was predominantly arteriosclerotic. Many of the small nerves in the pelvic fat were also surrounded by sarcoid-like granulomas.

In addition to the sarcoid-like granulomas with their predilection for perivascular sites, there were also the changes in the lungs that are characteristically found in asthmatics.[7] Excess mucus was found in the lumen of many bronchioles and in the mucus-secreting cells; the basement membrane of the bronchial epithelium was thickened, and there were foci of squamous metaplasia. Mast cells were numerous in the connective tissue about bronchi and bronchioles.

Comment

Many separate organ systems are involved in this case. Since it is always desirable in interpreting a case to attribute such changes to one cause, let us look for a common denominator. This one patient

* Courtesy of W. G. Kirchgessner, Bausch & Lomb Optical Company.

was unfortunate enough to have asthma, rheumatoid arthritis, focal pancreatitis, neuritis, arteritis, and glomerulonephritis, with terminal uremia. Histologically, there is a widespread sarcoid-like granuloma. The unusual feature is the marked dissolution of elastic tissue. While the antigenic nature of elastic tissue has not as yet been shown, it is a potentiality that is currently being investigated in this laboratory. The possibility is that all of the phenomena exhibited in this case may be explained by an antigen-antibody reaction and that elastic tissue may be the antigen.

The histologic pathology is presented in some detail because it is by study of the cell content, the inclusions, and the changes in the adjacent tissue that the morphologist postulates the actual mechanism of a process. Many studies have been made on the nature of the inclusions in the giant cells of granulomas.[12-15] In addition to these usual well-described inclusions, the granulomas in this case also presented small granules. The presence of granules has long been considered indicative of secretory activity of the cells containing them. In more cases than not, this has been proved correct. The presence of granules in the giant cells suggests that giant cells may be not only phagocytic but actively secretory as well. This leads to the possibility that by their activity an antigen may be released, which leads to autoimmunization.

Summary

A case of sarcoid-like disease with a distinct vascular component is presented. The clinical features were arthritis, asthma, hypertension, and visceral pain, with terminal uremia. The relation of the granulomas to the vasculature is described.

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Vascular Leiomyoma

A Study of Sixty-One Cases

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Cutaneous leiomyomas may be of one of two types, solitary or multiple, the two forms differing markedly in their clinical presentation and pathology. Multiple leiomyomas are usually solid tumors, but solitary ones may be either vascular or solid, with occasional tumors showing features of both. Formerly the two types of solitary tumor have been thought to be simple variants of the same neoplasm. However, after a study of 61 cutaneous vascular leiomyomas seen in the Laboratory of Pathology of the Harvard Cancer Commission from 1949 through 1958, we believe that a number of these tumors possess clinical and pathologic features which serve to establish them as entities distinct from other solitary cutaneous leiomyomas.

Despite a rather characteristic symptomatology, vascular leiomyomas do not appear to be well recognized clinically, and prior to this, Stout's 1937 review [6] contained the largest single group of them to be investigated; indeed, to our knowledge, no previous paper had been devoted exclusively to a study of these tumors. For these reasons we feel that our observations on this present group should be recorded.

Material and Methods

The records of 83 tumors with the diagnosis of "vascular leiomyoma" seen in the nine years prior to July, 1958, were extracted from the files of the Harvard Cancer Commission and the pathology and clinical histories of the cases reviewed. A questionnaire was prepared and circulated to the physicians who had originally submitted the tumors requesting follow-up data and, where necessary, additional clinical details.

It was decided to include only those cases in which the tumor was almost wholly composed of smooth muscle and in which the development of vascular channels with thick muscular walls formed a feature of the tumor. The number and size of such channels had to be greatly in excess of those needed for nutrition of the mass before the tumor was included in the study. Lack of clinical and follow-up information or failure of agreement in diagnosis caused 22 tumors to be discarded, leaving 61 tumors available for detailed study. Seven of these which showed some histologic features of either hemangioma or solid leiomyoma were felt to be borderline, but they showed sufficient morphologic resemblances to the main group of vascular leiomyomas to merit their inclusion.

Results

Age of Onset.—It was seen to be a tumor of middle life, by far the greatest number occurring in persons aged 30 to 50 years. No tumors were seen in the very young or the very old. A graphic depiction of the age distribution is seen in Figure 1. The length of time the tumor had been present or the

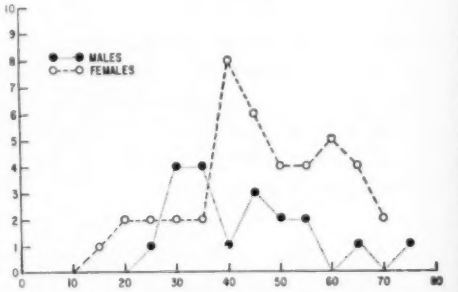

Fig. 1.—Age distribution of tumors. Abscissa: age of patients when tumors appeared. Ordinate: number of tumors appearing in each five-year period.

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The Laboratory of Pathology, Harvard Cancer Commission, and the Cancer Research Institute, New England Deaconess Hospital.

Distribution of Tumors by Age, Site, and Sex

Sex	Site	Ages						
		11-20	21-30	31-40	41-50	51-60	61-70	71-80
Males	Arising away from lower leg		3	1	3		1	1
	Arising in lower leg		2	4	2	2		
Female, single	Arising away from lower leg	2	1		1	1	1	
	Arising in lower leg	1		1	1			
Females, married	Arising away from lower leg		1			1	1	
	Arising in lower leg		2	9	8	7	4	

duration of symptoms varied from 2 months, in one case, to 25 years, in another. The greatest number of tumors (44) had been present less than five years, and of these, 18 had been present less than one year. The average length of time which elapsed between the patient's noticing the tumor and its removal was four years.

Sex.—Twice as many females as males appeared in the series, there being 41 females (one woman having two tumors) and 19 males; of the females, 32 were married and 9 were single. Figure 1 and the accompanying Table show that it is largely the married women who are responsible for the preponderance of tumors in the decades of 30 to 50 years. Unfortunately, detailed information concerning pregnancy, childbirth, and its complications is lacking for these women.

Site of Origin.—By far the greatest number of tumors arose in one or the other lower limb. Thus, 43 arose in or around the foot, the ankle, or the leg below the knee—23 on the left side, 20 on the right. Five arose in the thigh. The forearm was the next commonest region of origin, six arising there or on the wrist or hand. One tumor originated in the axilla; two arose in the lower lip; one each on the chin, the nose, the forehead, and the left temporal region (Table).

Clinical Features.—No cases of multiple tumors were seen, although one patient presented with two tumors, one of which had been present for 25 years, the other for 3 years. No constant concurrent condition could be detected, but one patient had radiation dermatitis of both lower legs, the tumor arising in the lower leg on the right side, while another patient had chronic dermatitis of both legs and the tumor arose on the medial side of the left lower leg. Seven tumors originated at the site of previous trauma and one at the site of a previous infective process. The most frequent complaint was simply of a tumor mass. In most cases this had grown slowly over a period of time, but some patients gave a history of rapid increase in size of the tumor and had had the mass removed because of this not long after its appearance. There was no correlation between other clinical and pathologic features and the rate of tumor growth. As many of the tumors were situated in the feet, several patients complained of trouble in shoe-fitting. The most striking clinical feature was pain, with or without associated tenderness. Twenty-seven of the tumors exhibited this symptom; 21 occurred in females, 6 in males; 25 arose in the lower leg, 2 (both in males) were situated elsewhere. The pain, which was usually described as being sharp or knife-like, was most frequently related to touch, often light touch, but in two cases the pain occurred intermittently in spontaneous fashion. In one case the pain was worse during pregnancy, relief being obtained by cold applications.

None of the tumors was diagnosed clinically as vascular leiomyoma. The following list shows the preoperative diagnoses that were assigned to the tumors:

Neurofibroma and fibroma	each	13
Cyst		9
Ganglion		7
Tumor, nature(?)		5
Lipoma, synovioma, hemangioma, sclerosing hemangioma, and fibrosis	each	2

Lymphoma, myoblastoma, mucocele, and bursa	each	1

Those tumors which exhibited symptoms of pain were diagnosed clinically as follows:

Fibroma		8
Neurofibroma		7
Cyst		3
Tumor, nature(?), and ganglion	each	2
Synovioma, lymphoma, lipoma, myoblastoma, and sclerosing hemangioma	each	1

Forty-five cases have had some form of follow-up, eighteen of these for five years or more. No instances of recurrence have been recorded following excision of the tumor. One patient has died of another condition, without recurrence, and 10 cases have no follow-up records. Four cases are too recent to be evaluated.

Pathology.—The whole group displayed a marked uniformity in size and gross appearance. They commonly arose in the deeper layers of the dermis, even in the subcutaneous tissue, and on occasion produced elevation of the overlying skin. Two tumors occurred in superficial situations just beneath the epidermis. They usually appeared as an encapsulated, glistening white nodule of tough fibrous tissue; a few tumors were bluish-gray in color; three were flesh-colored. Two tumors showed foci of calification. The majority were quite small, varying from 0.5 to 2.0 cm. in diameter, but one tumor attained a diameter of 3.5 cm. Menses and pregnancy had no effect on the size of the tumor; even in the one case where pregnancy appeared to produce spontaneous pain in the tumor, no change in size was noted. The color of the overlying skin was variously recorded as being reddish-brown, bluish, or pink, the degree of dilatation of vessels in the region at the time of observation probably being responsible for these changes.

Microscopic Appearances.—Almost all the tumors showed a well-defined fibrous capsule; only the two tumors which were situated superficially showed spread of plain muscle bundles into the surrounding tissues. In all cases the bulk of the tumor consisted of plain muscle bundles distributed in random fashion throughout the mass. In many places these muscle bundles were condensed into tight whorls, an arrangement that was most marked about the many vascular channels which traversed the tumors (Fig. 2). Frequently, dense bundles of muscle would fuse with and become part of the wall of a vessel, producing the appearance of a mass composed of many thick-walled blood vessels (Fig. 3). Where this type of arrangement was seen, the enclosed vascular space was usually small and slit-like. However, there were often other vascular spaces which were widely dilated, and these vessels appeared to have comparatively little plain muscle in their walls (Fig. 4). Elastic tissue stains revealed true elastic laminae in the nutrient arterioles of the neoplasms, but the great majority of the vessels in the tumors showed no elastica in their walls. In certain cases the origin of the tumor could be traced to small vessels in the adjacent dermis; a few appeared to arise from arterioles, while some appeared

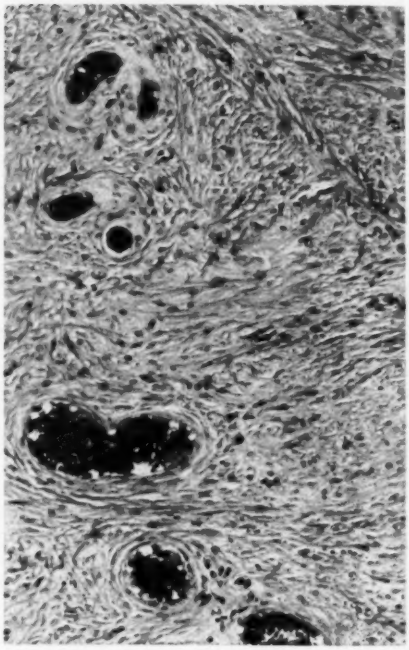


Fig. 2.—Tumor traversed by bundles of smooth muscle, which are condensed about the vascular channels. Hematoxylin and eosin; × 150.

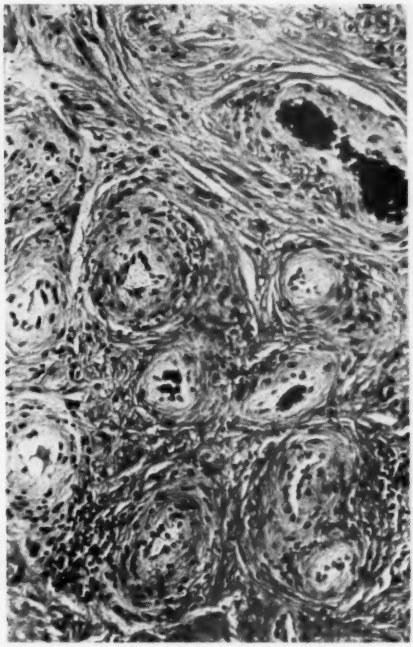


Fig. 3.—A collection of thick-walled blood vessels. Note that the smooth muscle bundles of the rest of the tumor fuse with and become part of the vessel walls. Hematoxylin and eosin; × 150.

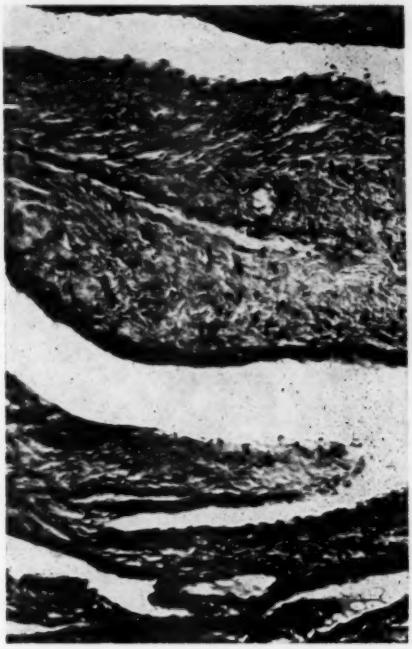


Fig. 4.—Portion of tumor showing dilated vascular channels with little muscular thickening of their walls; Van Gieson stain; × 150.

to arise from the walls of veins. In many the exact point of origin could not be defined.

In addition to nonstriated muscle fibers, the stroma of most of the tumors contained varying amounts of collagen, and in several cases portions of the tumor had undergone myxomatous degeneration (Fig. 5). This degenerative process was seen commonly in those tumors giving rise to pain.

As the symptom of pain was seen in 44% of the cases, we thought that the tumors might contain large numbers of nerve fibers. Previously, Stout [6] and Jansen [3] had attempted to demonstrate such structures in vascular leiomyomas without success. Toned and untoned Bodian-stained sections showed either very few or no nerve fibers in the 12 tumors examined by us. The tumors in the borderline groups showed essentially the same histologic pattern as the main group, and only variation in the numbers of vessels which they contained served to distinguish them. Some infiltration by either acute or chronic inflammatory cells was seen in 30% of the neoplasms.

Comment

The most striking thing about these tumors was their curious age, sex, and site distribution. Thus, 40% of them appeared in married women of ages between 30 and 60 years, and 70% arose in one or the other lower leg. This type of distribution of cutaneous leiomyomas has not previously been reported; but as nearly all prior reports have included solid as well as vascular tumors, and frequently examples of the multiple forms as well, it would not be surprising if small variations in the distribution of the vascular type of tumor were overlooked. In this study vascular leiomyomas, or angioleiomyomas, have appeared as a separate group distinguished from other

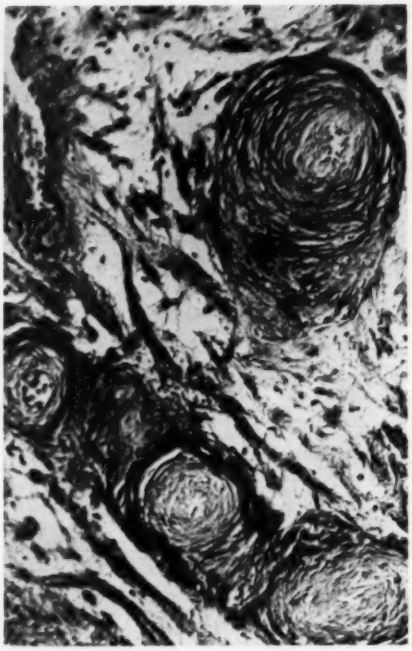


Fig. 5.—Myxomatous degeneration in a tumor. The smooth-muscle thickening of the walls of some of the small vessels is prominent. Phosphotungstic acid hematoxylin; × 150.

forms of leiomyoma not only by their possession of abnormal numbers of vascular channels but also by this unusual age, sex, and site distribution.

In attempting to explain this unusual distribution, it was noted that it was just those decades—30 to 60 years—in which leiomyoma of the uterus is most commonly found. This led us to suspect the role of pregnancy and sex hormone levels in the pathogenesis of some of our tumors. During pregnancy there is frequently some obstruction to the venous return from the lower leg, with resultant venous stasis in that region. In certain cases this results in clinically manifest varicose veins; in others a temporary tortuosity of the veins of the lower limb is the outcome. These deformities of the vascular system of the leg are notoriously prone to damage by minor trauma and infection, and it has seemed to us that such a focus of irritation or infection, under the influence of estrogenic hormone, might well produce a mass composed of plain muscle, collagen, and many vascular channels. Support for such a concept is seen in the work of Lipschütz,[4] who has produced estrogenic neoplasia at the site of foreign-body reactions. He says:

> Localization of experimental fibroids at angular sites or in sites of mutual contact necessarily suggests an unspecific mechanical influence. Such an influence has been shown to be real. . . . In males in which a ligature was made on the ductus deferens, tumors were elicited at the site of the ligature when estrogens were given. These tumors were the most typical fibromyomas or myomas which we have met with in our work with estrogen-induced fibroids.

It should be noted that most of Lipschütz' experiments have been conducted with the guinea pig, an animal in which the connective tissues are extremely labile; consequently, his findings must be accepted with some reserve. However, similar views have been advanced by Burrows and Horning[1] in relation to trauma, infection, and estrogen-induced hyperplasia. They point out that estrogens become fixed and concentrated in tissues that are inflamed, and that they increase the rate of mitosis in those parts. In the present series it is of interest to note that eight patients gave a definite history of either previous trauma or infection occurring at the site of origin of the tumor, while two patients had dermatitis in the leg in which the tumor arose.

Further support for this hypothesis of the origin of some of these tumors is seen in their close morphologic relationship to dermal hemangiomas. Many of the hemangiomas seen in the skin in older persons represent simple reactions to injury of one sort or another, disappearance of the initial inflammatory reaction leaving a small mass of new vessels with much new fibrous tissue. It requires little imagination to visualize a vascular leiomyoma in some of these foci if the proliferating connective tissue were nonstriated muscle rather than fibrous tissue. As we have pointed out above, the differentiation between an angioma with thickening of the smooth muscle of the vessel

walls and a vascular leiomyoma with many widely dilated blood vessels within it may well be impossible. Indeed, vascular leiomyomas may represent merely one point in a process of continuous proliferation of smooth muscle, which could be represented graphically as is shown at the bottom of this page.

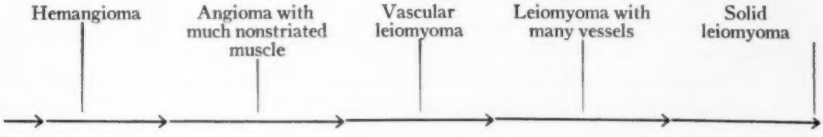
Of course, a concept such as this suggests that an appreciable number of vascular leiomyomas may not be true tumors but vascular malformations containing hyperplastic nonstriped muscle. Thus it is unfortunate that the obstetric histories of many of our patients are lacking and that now we are unable to obtain specific details concerning instances of minor trauma and infection, for these omissions prevent us from reaching definite conclusions on our suggested mode of origin of certain of these tumors. However, wider appreciation of the existence of vascular leiomyomas should help us to acquire more accurate information about them, and this may provide us with the answer to their pathogenesis. Such information may also be of use in the wider field of hormone-dependent tumor growth.

If we are to obtain more details of the life histories of these tumors, clinical recognition of them will have to be improved. In none of our present cases was the diagnosis made clinically, and even in those cases in which spontaneous pain was experienced the most favored preoperative diagnoses were neurofibroma and fibroma. As Stout [7] has shown, these latter tumors are only very rarely spontaneously painful, while vascular leiomyomas are probably the tumors which exhibit this symptom most frequently; the only other cutaneous tumor which commonly gives rise to pain spontaneously is the glomus tumor, and in our laboratory it is only one-third as common as vascular leiomyoma. From our experience in this study, we feel that a nodule arising in the lower leg of a woman aged between 30 and 60 years which gives rise to spontaneous pain will almost invariably prove to be a vascular leiomyoma.

The cause of the painful symptoms remains unknown. Large numbers of nerve fibers could not be demonstrated in the tumors in our series, and the most likely explanation would seem to lie in contraction of the tumor vessels, producing ischemia within the muscle mass. Histologic evidence of degenerative changes in a number of the tumors which did exhibit symptoms of pain suggests that this might be so.

In view of the inability of classic histologic methods to demonstrate the precise site of origin of many cutaneous leiomyomas, the studies of Senter [5] are of interest. He was able to control the occurrence of spontaneous pain in the tumors of a case of multiple cutaneous leiomyomas by the use of a number of drugs; the pharmacologic reactions suggested that the smooth muscle of the tumors was derived from that of sweat glands. By a similar method Japanese workers [2] were able to demonstrate the probable origin of certain other of these tumors from the pilomotor muscles. In those tumors which do give rise to pain, the type of smooth muscle of origin of the mass would seem to be indicated more precisely by a procedure such as this than by a study of stained sections.

The most satisfactory treatment of these tumors is complete excision. All the patients in our series were relieved of their symptoms by removal of the mass, and no evidence of recurrence has been noted in those cases which have been followed for more than five years. As these tumors are prevalent in women during and just after the childbearing period, second tumors might well arise in some cases. In one of our present patients this did in fact occur, and,



although both tumors occurred in the right lower leg, they were quite distinct and appeared at widely separate times. If surgery were impracticable for any reason, pharmacologic control of painful symptoms in the manner suggested by Senter [5] might be attempted.

Summary

The pathology and clinical manifestations of 61 cases of vascular leiomyoma are reviewed.

An unusual age, sex, and site distribution of these tumors is seen and its significance discussed.

The hypothesis is advanced that the distribution of certain of these tumors is determined by mechanical factors and estrogen levels in the tissues, and that consequently some vascular leiomyomas may in fact be malformations and not neoplasms in the true sense of that term.

It is suggested that in some cases pharmacologic control of the pain felt in the tumors might be a more precise indicator of their site of origin than classic histologic methods.

We are indebted to Dr. S. P. Hicks and Miss M. A. Coy, A.B. for performing the Bodian stains and to Dr. Olive Gates for permission to use material from some of the earlier cases originally diagnosed by her.

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Vascular Lesions in Rats with Nephrotoxic Renal Disease

Relation to Secondary Hyperparathyroidism

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Chronic renal disease simulating the nephrotic syndrome as seen in infants and children can be produced in rats by intravenous injections of rabbit anti-rat-kidney serum (NTS).[1] This results in a frequently progressive disease, with eventual renal failure. The course of the disease is characterized by marked proteinuria, hypoproteinemia, anasarca, hyperlipemia, and, if progressive, by terminal elevation of blood urea nitrogen and a variable degree of hypertension. The present report is designed to evaluate the relation of these factors to the development of lesions of the vascular system.

Methods

Renal disease was produced in Sprague-Dawley rats by the intravenous injection of rabbit anti-rat-kidney serum, as previously described in detail.[1] Studies were done on 65 rats that developed chronic progressive renal disease with terminal renal failure ("uremic" group). The animals were killed at intervals of 1 to 14 months after the administration of NTS. Thirty-five additional rats were killed less than a month after the injection of NTS. These latter animals did not have signs of marked renal failure, and served as "controls." All animals were fed a diet* containing approximately 5% fat, 24% protein, and 42% carbohydrate.

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* Purina Laboratory Chow, Ralston Purina Co., St. Louis.

Heart blood was drawn at the time of killing and was analyzed for urea nitrogen,[2] creatinine,[3] total lipids,[4] cholesterol,[5] serum protein,[6] and, in 20 cases, calcium[7] and phosphorus.[8] The blood pressure was determined at intervals by the method of Kersten and associates.[9] Paraffin-embedded kidney sections were cut at 4μ and were stained with hematoxylin and eosin and by the periodic acid-Schiff method (PAS); sections of heart, aorta, and parathyroid were stained with hematoxylin and eosin; in 34 cases, sections of aorta were stained with toluidine blue and by the PAS method. Frozen sections of heart, aorta, and kidney were stained for fat with Sudan IV.

The formalin-fixed parathyroid glands of 20 rats with chronic renal disease and those of 18 normal control rats were measured with a dissecting microscope and a micrometer eyepiece. Two perpendicular diameters were measured for the two parathyroids in each rat, and the average was taken as an index of the size of the glands.

Results

The kidneys of all 65 rats with uremia showed a microscopic picture of marked chronic disease (Fig. 1). There was fibrosis of most of the glomeruli, and extensive infiltration of the interstitial tissue with lymphocytes and plasma cells. The arteries were thickened and contained intramural lipid in 13 animals and medial calcific deposits in 22 animals. Calcium was found in the renal arteries only in rats which also had calcium in the aorta and coronary arteries, with but two exceptions. This relation did not hold for fat, and there was no correlation between the presence of fat in the renal vessels and of fat in the aorta or coronary arteries. Calcium was also deposited in the interstitial tissue of the kidneys. It was most prominent around the convoluted tubules and seemed to involve the tubular basement membrane.

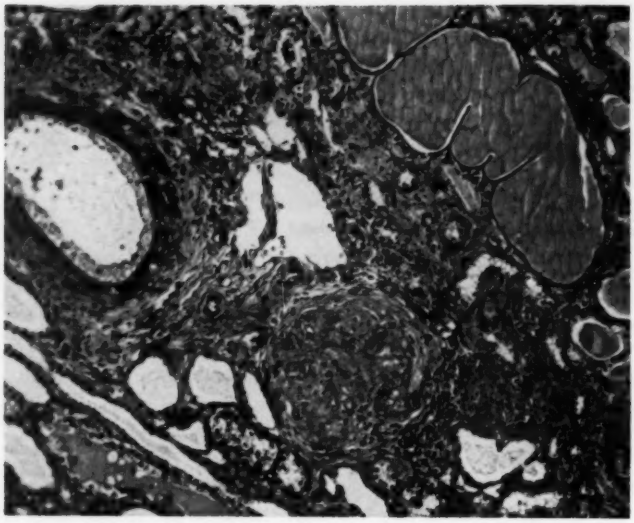

Fig. 1.—Marked chronic renal disease. Note fibrous obliteration of glomerulus, dilated tubules with hyaline casts, interstitial fibrosis and chronic inflammation, calcification around some tubules, and calcification and intimal thickening of small artery. Hematoxylin and eosin; reduced to 83% of mag. × 155.

The aortas and coronary arteries of 44 of the 65 uremic rats had lesions containing either calcium or fat or both. The aortas of 39 rats contained deposits of calcium in the media, which were visualized microscopically and, in many instances, in roentgenograms. In the more severely involved aortas calcium was present in concentric bands and resulted in a "trachea-like" appearance, similar to that illustrated by Rather [10] for a series of rats on which various renal operations had been performed.

Microscopically, the basophilic material that was seen in hematoxylin-stained sections (Fig. 2) was positive in von Kossa preparations. It was deposited between and adjacent to the elastic lamellae in the aortas with slight involvement; but with more marked involvement the elastic tissue was

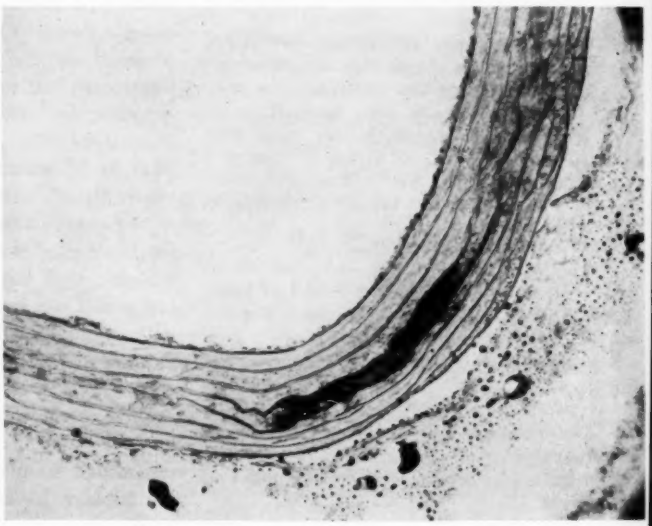

Fig. 2.—Medial calcification of aorta. Dark-staining material deposited along and between elastic lamellae was positive for calcium with the von Kossa technique. Frozen section stained with Sudan IV and hematoxylin. No fat is present in aorta, although blood cholesterol was 400 mg. %; total lipids were 3,220 mg. %, and blood pressure was 208 mm. Hg. Reduced to 80% of mag. × 155.

replaced by the basophilic material, and in some instances almost the entire media was distorted and replaced by the calcific deposits. Similar calcific lesions were present in the coronary arteries of 30 rats. Although there were, therefore, nine rats which had aortic calcification without coronary artery involvement, coronary artery calcification was not seen without simultaneous aortic involvement. Sections through the upper legs of 15 rats with aortic calcification revealed 7 instances in which there was calcification of the popliteal artery or its branches.

Lipid deposits were found in the arteries of 18 rats. In contrast to the commoner appearance of calcific lesions in the aorta, lipid deposits were more marked and more commonly found in the coronary arteries (14 rats) than in the aortas (8 rats). In a few instances the fat droplets were confined to macrophages in the intima, but more commonly the fat was rather diffusely spread through the intima and media (Fig. 3).

No correlation could be found between the calcific medial lesions and the lipid deposits in arteries. Five rats with lipid deposits did not show any areas of calcification, and 26 rats had extensive medial calcification without concomitant lipid deposition. Thirteen rats showed both types of vascular lesion, but even here the lipid deposition was only rarely in a region that also had medial calcification. Furthermore, no correlation could be found between either the calcific or the lipid-containing lesions and the blood pressure, or any correlation with the total lipid or the cholesterol blood level, or with the sex of the animal.

In most of the 34 aortas of uremic rats that were stained with toluidine blue and by the periodic acid-Schiff method, some metachromatic and PAS-positive material was found between and adjacent to the elastic fibers. The amount of this material was roughly proportional to the blood urea nitrogen level.

The parathyroid glands of 20 of the "uremic" rats were found to be significantly larger than those of 18 control animals. In Figure 4, average diameters of the parathyroids are plotted against the body weights of the animals. The values for the "uremic" animals are seen to be well outside the normal range in almost all instances. The parathyroid diameters average 3.03, with a standard error of ± 0.14 in the control group, and 4.97 ± 0.29 in the

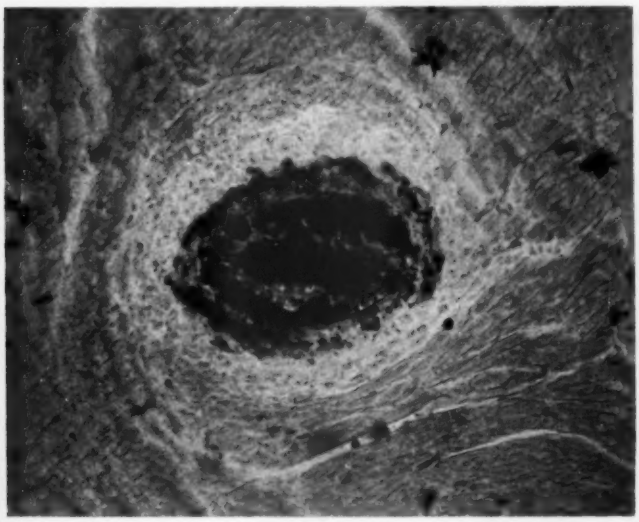

Fig. 3.—Coronary artery. Fat droplets in thickened intima are mainly within macrophages; in media fat is diffusely distributed. Sudan IV and hematoxylin; reduced to 83% of mag. × 385.

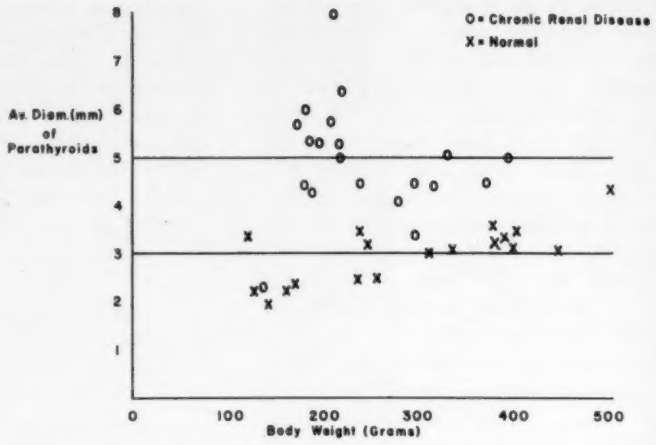

Fig. 4.—Effect of renal failure on size of parathyroids. The lower horizontal line represents the mean value for the normal rats, and the upper line represents the mean value for rats with chronic renal disease. Figures on ordinate are 3.5 times the true value in millimeters.

"uremic" rats. This is a significant difference, with a probability of less than 0.001 that the observed difference could be due to chance. In addition to the increased size of the parathyroid glands in the "uremic" rats, microscopic examination of the glands revealed a difference between the two groups (Fig. 5). The cells in the "uremic" group were often more closely packed and more granular, and frequent mitoses were seen, in contrast to the more vacuolated cells with only rare mitotic figures seen in the control group.

Focal myocardial necrosis was found in 45 of the 47 rats with renal failure, which also had vascular lesions, and in 11 of the 18 rats with renal failure which did not show any vascular lesions. A few similar, but less severe, lesions were found in the hearts of 4 of the 35 control rats.

All of the 65 "uremic" rats had a blood urea nitrogen of over 80 mg. %, and the level was actually less than 100 in only 2 rats. The blood creatinine in these rats was above normal, varying from 2.5 to 8.4 mg. % (normal, up to 1 mg. %); the

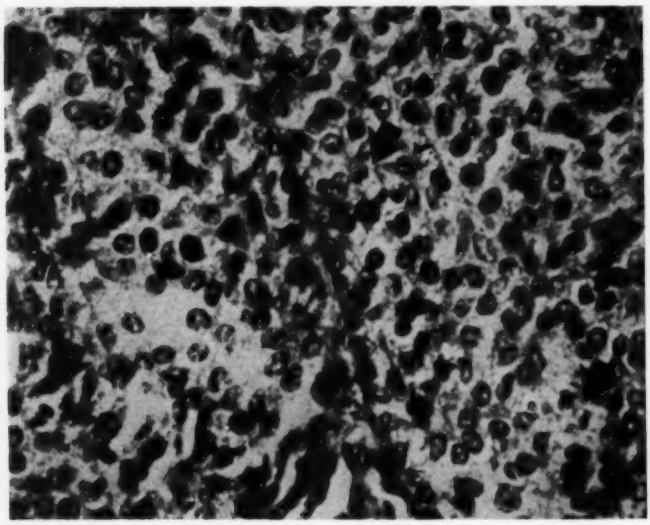

Fig. 5. — Parathyroid gland from rat with chronic renal failure. The gland is enlarged, as determined by direct measurement. Note three mitotic figures. Hematoxylin and eosin; reduced to 83% of mag. × 580.

total lipid levels in the blood varied from 440 to 3,200 mg. % (normal, up to 400 mg. %), and the blood cholesterol values varied from 89 to 400 mg. % (normal, up to 100 mg. %). The blood calcium and phosphorus levels were abnormal in all of the 20 uremic rats in which these substances were studied. The product of the concentration of calcium and phosphorus was high in all cases—as high as 312 in one rat. The phosphorus level was also markedly elevated in all instances, varying from 10 to 32 mg. %. The blood calcium level was within the normal range of 9 to 11 mg. % in most instances but was less than 7 mg. % in four rats, and was between 12 and 15 mg. % in three rats.

The control group of 35 rats all received a dose of active NTS similar to that received by the "uremic" group, but they were killed less than a month after the injection and had not yet developed evidence of marked renal failure. The blood urea nitrogen was less than 80 mg. % in all animals, varying from 23 to 78 mg. %. The terminal blood cholesterol and total lipid values were elevated in most of these rats to levels comparable to those of the "uremic" rats, with the cholesterol varying from 76 to 326 mg. % and the total lipids from 370 to 2,450 mg. %. Nevertheless, none of these rats had any lipid or calcific deposits in their aortas or coronary, renal, or popliteal arteries.

Comment

It is reasonable to postulate that the calcific vascular lesions that are seen in this study may be related to increased parathyroid activity secondary to renal failure. More direct evidence for this assumption could be provided by controlled studies with parathyroidectomized animals; such studies are planned. The present experiment, however, provides some evidence for this concept. The blood phosphorus level is markedly elevated in all rats in the "uremic" group. The high blood calcium levels in 3 cases suggest increased parathyroid activity, and the normal values in 13 cases were obtained in spite of decreased plasma protein concentration, and thus are also in accord with an assumed increased activity of the parathyroid glands. Finally, direct measurements of diameters and histologic observations of the parathyroid glands show them to be hypertrophied, and probably hyperplastic, in the "uremic" animals.

Calcific vascular lesions similar to those observed in the present study have been produced by other workers.[10-13] Lehr and Churg[11] produced renal injury with a poorly soluble sulfonamide, with subsequent cardiovascular necrosis and calcification. These effects could be prevented by a preceding thyroparathyroidectomy. Wilgram and associates[13] produced calcific vascular lesions by giving rats a choline-deficient diet supplemented by cholesterol. They felt that the cardiovascular lesions were probably etiologically linked to the renal damage induced by the acute choline deficiency.

The observation of increased amounts of PAS-positive, metachromatic material in the aortas of the "uremic" animals may be of importance in understanding the pathogenesis of the calcification. Several other studies have indicated that injury to the ground substances of vessels may play an important part in the early stages of arteriosclerosis.[14] Damage to vessels in rats by allylamine[15] and by feeding toxic Lathyrus factor[16-17] have been accompanied by increased PAS-positive material and (in the latter situation) by increased metachromasia. It has been shown by Engel[18] that the administration of parathyroid hormone results in an increase in the blood level of seromucoid, and he suggested that the hormone caused depolymerization of bone matrix, so that mucoprotein components diffused out. It is possible that the hormone has a similar effect on the blood vessel wall, and that the acid mucopolysaccharides which appear in the media of the experimental animal provide the "local factor" substrate for deposition of calcium salts. In the presence, then, of the high calcium-

phosphorus ratios in the "uremic" animals, calcification could take place. This agrees with the findings of Rubin and Howard,[19] who believe that acid mucopolysaccharides play a specific role in the calcifying mechanism.

In previous studies in which arterial calcification in rats was produced with excess dihydrotachysterol (AT-10),[20] or in which myocardial calcification was produced with hydrocortisone,[21] testosterone seemed to have a protective effect. In the present study, however, no relation is noted between the sex of the animal and the deposition of either fat or calcium in the aorta or heart.

The lipid-containing lesions of the aorta and coronary arteries in our experiments are similar to the "lipomatous" and "atheromatous" lesions that Wissler et al.[22] produced in rats by diets containing a high choline content, and are also similar to those produced by Hartroft and co-workers,[23] who gave rats high-lipid, low-protein, and low-choline diets. Like Wissler, we found that the fat-containing lesions were more marked in the coronary arteries than in the aorta. Wissler found that the lipid deposits were increased when the rats were subjected to a "hypertensive regimen," consisting of injection of a nephrotoxic serum plus desoxycorticosterone acetate. However, he did not find a correlation between the degree of hypertension and the severity of the lesions. In our studies, the degree of hypertension also could not be correlated with the amount of lipid deposition. Wilgram, Lewis, and Blumenstein[24] found coronary "lipidosis" in rats on a choline-deficient diet in the presence of low blood cholesterol and lipoprotein levels. Wissler et al.[22] also found that rats with the highest levels of blood cholesterol did not have more marked lipid deposits in their coronary arteries. A similar lack of correlation is found between the level of hypercholesteremia and the degree of fat deposition in our rats. In contrast, Deming and co-workers[25] found a positive correlation of the extent of atherosclerotic lesions in rats, the concentration of cholesterol in their serum, and the height of the blood pressure.

The "filtration theory" of human atherogenesis, if applied to the rat, would suggest that the occurrence of changes in the medial ground substance should predispose to the deposition of lipids. Recent studies in the rabbit[26] are compatible with this concept. The present experimental sudies are well adapted to evaluate the various factors involved in the rat, since various degrees and combinations of medial damage and lipid deposition are present, together with various degrees of hyperlipemia, hypercholesteremia, and hypertension. However, no correlation can be observed between either the height of the blood pressure or the height of the blood lipid levels and the amount of lipid deposited in the aorta or coronary arteries. In addition, there is no correlation between the sites of medial injury and the sites of fat deposition. The implication of these observations is that the medial calcific lesions and the lipid-containing lesions, as produced in rats by the present experimental procedures, are largely independent of each other.

Summary and Conclusions

Chronic renal disease leading to severe uremia was produced in 65 rats by the injection of rabbit anti-rat-kidney serum (NTS). Study of the arteries showed calcific medial lesions in 39 rats and lipid deposits in 18 rats. No vascular lesions were noted in 35 additional rats that had been injected with NTS but had not developed similar degrees of renal failure.

No correlation was found between the presence of the calcific medial lesions and the lipid deposits, and it was suggested that the two phenomena are independent of each other. In addition, there was no correlation between the arterial lesions and the level of blood lipids, the level of the blood pressure, or the sex of the animal.

Evidence was presented that indicates the existence of secondary hyperparathy-

roidism in the rats with chronic renal disease and marked renal failure. These animals had increased diameters of their parathyroid glands, marked degrees of hyperphosphatemia, and increased calcium-phosphorus products, as well as an increased amount of PAS-positive and metachromatic material (probably acid mucopolysaccharides) in their aortas.

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Striated Annulets (*Ringbinden*)

Their Experimental Production in Mammalian Muscle

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Striated annulets (*Ringbinden*, ringed fibers) occur when the peripheral fibrils of a muscle fiber run spirally, encircling the central, longitudinally running fibrils. Since the discovery of these structures by Bataillon, in 1891, more than 50 reports have appeared in the literature, some of them in English.[2-7] Besides occurring naturally in normal and diseased muscle, annulets have been produced experimentally—in Amphibia by Doms[8] and by Weiss and James[5] and in Mammalia by Vecchi[9] and by Goerttler.[10]

Vecchi encountered annulets when he was studying the effects of detaching a muscle (the sternomastoid of the white rat) from its origin and insertion. To prevent the muscle acquiring new attachments, he sewed the ends together, to form a muscle ring. Rats were killed at increasing intervals postoperatively, those which survived six months or more showing annulets in the muscles operated on, whereas there were none in the controls. Repeating this work, Wohlfart found annulets four months after operation.[11]

It was decided to repeat Vecchi's work, but to use the New Zealand white rabbit (Oryctolagus cuniculus) instead, and to try to find out as much as possible about the annulets.

Material and Methods

The sternomastoid complex of the rabbit consists of three parts: a lateral cleido-occipital, a medial sternomastoid proper, and a deep cleidomastoid, or basioclavicularis. The right cleido-occipital was operated on, the left being used as the control. The animals were four to six months old at the time of operation.

Standard Procedure (Thirteen Rabbits).—After the muscle was identified, the upper and lower ends were freed and cut away from their attachments. In the first two rabbits operated on, the muscles were cut somewhat short of their insertions, but afterward the cuts were made right on the bone. The lower end was further mobilized to bring the muscle ends together. They were then tied into a ring with silk thread. Arterial bleeding near the upper end of the muscle and venous bleeding from the large veins on its outer surface made the operation difficult.

Defects in Technique.—The use of silk was found to have been ill-advised, for it caused marked fibrosis, which obscured the operative field. It was usually impossible to remove the thread afterward, and it interfered with section cutting, so much so that the specimen had to be discarded in one case, while it was considerably damaged in another. Difficulty in identifying the operative specimen led to the exclusion of three cases.

Modified Operations.—In two rabbits operations to test the effect of steps in the operative procedure itself were done. In one animal, the muscle ends were detached but no circle was made; in the other, the major blood vessels were divided, but the muscle was otherwise left intact.

The animals were killed from 2 to 7 months and 10 days after operation (Table). The muscle operated on was removed and placed in fixative. Nine standard operative specimens and the two modified operative specimens appeared suitable for study.

The left cleido-occipital muscle was cut from its attachments, stitched to cardboard in an extended position, and placed in fixative, but only three specimens were examined histologically.

The tissues were fixed in a mixture of 2 parts of 10% formalin and 1 part of 5% trichloroacetic acid, as recommended by Wohlfart.[8] After 45 to 48 hours they were dehydrated, cleared in xylene, and embedded in paraffin. Sections (10μ) were cut transversely, except for one specimen, in which they were cut longitudinally. Staining techniques included hematoxylin and eosin and Bodian's silver

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This paper is based on a New Zealand University Doctor's Thesis.

Summary of Annulet Production

Survival After Operation	No. of Annulets Counted in			Comment
	1:40 Sections	Serial Sections	Opposite Muscle	
2 mo.	--	19	Nil	
2 mo. 18 days	77	--	--	
3 mo. 26 days	162	--	--	
4 mo. 25 days	33	--	--	Area containing annulets damaged; figure probably too low
5 mo. 14 days	--	4	Nil	Muscle not divided close to ends and part of "dead-space" area of block lost
5 mo. 22 days	388	--	--	
6 mo. 21 days	70	--	--	Muscle cut in long section; so figure is not strictly comparable
7 mo. 4 days	521	--	Nil	600 annulets counted in 1,000 serial sections; 441 counted in same area at 1:40 sections
7 mo. 10 days	1	--	--	Muscle divided farther from ends than in others
4 mo. 17 days	Nil	--	--	Major blood vessels only divided
4 mo. 17 days	Nil	--	--	Ends detached but no ring made

stain. Serial sections were examined in the three control specimens and in two of the operative specimens. In the remaining cases approximately 1 in 40 sections was mounted in the first instance. Slides from four of the specimens were projected and drawn to work out their over-all arrangement.

Results

Control and Modified Operative Material.

No annulets were seen in the three left cleido-occipitals examined, in the muscle which had merely been devascularized, or

Fig. 1.—Group of annulets. Bodian stain; × 1,100.

in the muscle in which the ends had been divided but no loop made.

Appearance of Experimental Muscles.—It was very difficult to interpret the specimens in terms of the simple loop made at operation. There were, however, two fairly reliable guides to the layout of the muscle—the suture line and the dead space—a term used here to refer to the region which lies enclosed within the muscle loop.

Histologically, at least three types of tissue could be identified. First of all, there was normal muscle, presumably corresponding to the bulk of the muscle belly. Then there was a zone containing small patches of muscle, the pattern of which changed rapidly in succeeding sections. This zone probably represented the original dead space into which both single fibers and small fasciculi appeared to have separated from the main muscle mass. In most of the blocks this area was infiltrated with fat. Intermediate types of tissue were also found with medium-sized fasciculi. Finally, there was a large zone around the suture line where there was much fibrosis and irregularity of the muscle fibers, both in direction and in caliber.

Distribution of the Annulets.—More than 50% of the annulets occurred in the dead space, being confined to this area in three rabbits. In the others, annulets also occurred in normal muscle. Such annulets nearly always occurred singly, and they frequently lay at the edge of their fasciculi. In the dead space they occurred either in isolated fibers or in small fasciculi, which often contained several annulets (Fig. 1).

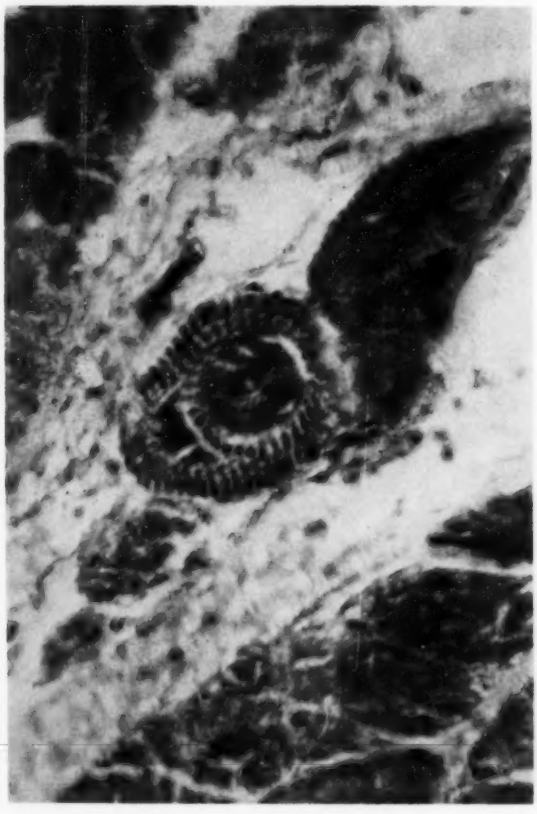

Fig. 2.—Annulet with intersecting fibrils—a complicated annulet. Note the thin annulet next to it. Bodian stain; × 1,100.

Sometimes medium-sized fasciculi were found containing numerous annulets. The suture line tissue, however, contained very few. In general they did not occur at the ends of the blocks, their peak incidence being about the middle of the blocks. Unfortunately, it was not possible to decide whether or not the annulet peak was at the site of nerve entry.

Incidence of the Annulets.—The accompanying Table shows the number of annulets counted in either serial or representative sections of the muscles operated on and their complete absence in the control material. From this Table it is clear that a few annulets are present in the animal which survived from two months after operation; and it would appear that the number of annulets increases as the survival time is lengthened, but that there are certain exceptions which are considered more fully in the Table.

Morphology of the Annulets.—The annulets resemble in size those described by other authors. Very thin annulets were seldom seen in the rabbit which was killed seven months after operation, but they were present in most of the other specimens. On the other hand, all the annulets present in the two-month animal seemed fairly thick. Complicated annulets were also seen in which fibril bundles intersected the axial fibrils (Fig. 2). One such complicated annulet was seen in the two-month specimen. In some fibers a few fibrils lay outside the annulet, and one or two annulets lay within the fiber. A striking feature of a number of beautiful annulets—which lay among normal muscle fibers in many cases—was

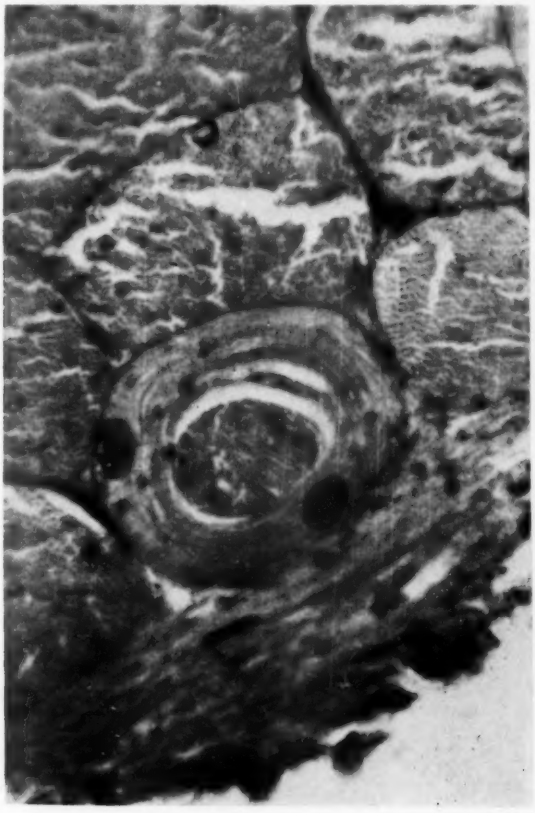

Fig. 3.—Annulet showing two nuclei. Only the Z-disc is apparent. Hematoxylin-eosin stain; × 1,100.

the presence among the circularly running fibril bundles of normally shaped nuclei (Fig. 3).

Comment

My results confirm the findings of Vecchi [9] and Wohlfart [11] that striated annulets develop if the ends of a muscle are cut and tied together, the nerve supply being left intact. However, after an interval of only two months annulets appeared in one rabbit, two months earlier than with Wohlfart and four months earlier than with Vecchi. This may have been a species difference—since the previous work was probably all done on rats—or more likely it was because I examined the whole of the specimen, either in serial or in reasonably close representative sections.

It appears that the number of annulets in general increases significantly with the length of the survival time, although there are certain discrepancies in the results. Unfortunately, it is not possible to correlate changes in fiber direction with annulet formation. It is clear, however, that in this procedure, although many annulets do occur in normal muscle, more occur in loose tissue, where muscle fibers either lie singly or else in small fasciculi. Nevertheless, the annulets resemble those described by other workers in shape, size, and presence of complicated forms and of nuclei among the circularly running fibrils.

Significance of Annulets

It is now generally considered that annulets are genuine structures, although

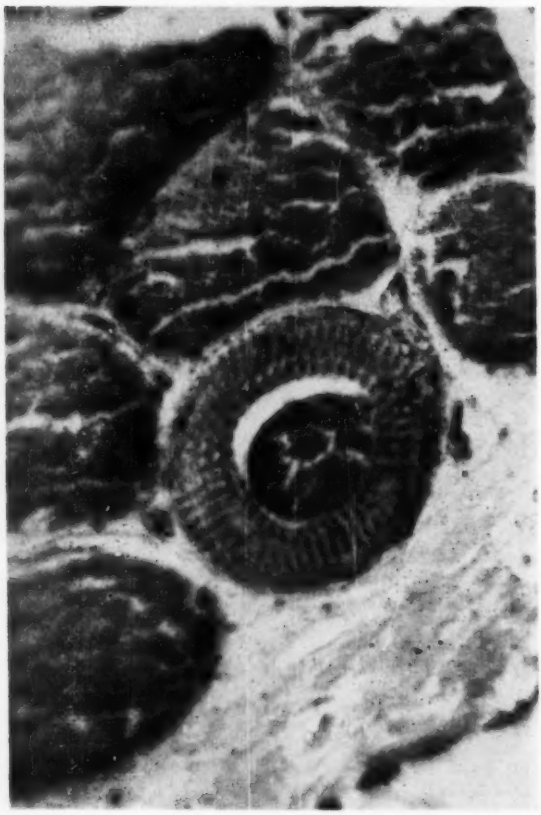

Fig. 4.—Same annulet as in Figure 3. Note that the cross striation of different fibril bundles is not in register and that the cross striations meet at an angle. Bodian stain; × 1,100.

Adams, Denny-Brown, and Pearson [12] support the view of Schaffer [13] that annulets are artifacts. Schaffer's arguments are, however, unconvincing.

On the other hand, Heidenhain,[14] Schwarz,[15] and Graf [16] consider that annulets have a useful role to play, but it is difficult to imagine what this could be in a muscle circle! The increasing occurrence of annulets in eye muscles with age, as shown, for example, by Bucciante and Luria,[17] and their presence in the tail muscles of metamorphosing Amphibia [1] and in the postmature axolotl,[18] suggests that they may be merely a manifestation of aging. Bucciante and Luria, morever, point out that disease often simulates aging processes. This may explain their presence in diseased muscle. However, the actual amount of activity, not merely the duration of activity, as in old age, may be the key to annulet formation—a view which is strongly supported by Siliotti's finding many more annulets in a squinting muscle than in those acting normally.[19] Goerttler,[20] however, believes that annulets arise in muscle in response to an abnormal functional burden.

While the results of this experiment are inconclusive, they certainly show that annulets are not artifacts. The increase in the number of annulets with time supports the view that they represent an aging process, whereas their presence mainly in loose tissue might indicate that muscle fibers develop annulets as a result of changes in their environment.

Summary

Striated annulets (*Ringbinden*) may be produced by detaching the rabbit cleido-occipital muscle from its attachments and sewing the ends together to form a ring. Annulets are present two months after operation, and they increase in number the longer the postoperative period. Although some occur in normal muscle, more than half are found in isolated fibers or small fasciculi. It is uncertain whether muscle fibers develop annulets as the result of aging processes or because of changes in their environment.

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Auxiliary Method for Study of Atherosclerotic Lesions

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Pathologists, histologists, chemists, biochemists, physicians, and statisticians must combine efforts to elucidate the development and progress of atherosclerotic lesions. Area involvement and character of alterations (yellow streaks, white patches, combined and complicated lesions) are the major problems when large blood vessels are studied. The prime problems in the study of small arteries are localization of lesions and encroachment upon the lumen. Intricate but important problems are tackled through microscopic and chemical investigation. Acceptable methods for grading of lesions are needed to learn about the life history of the disease and provide statistically valid data about the incidence and degree of atherosclerosis.

A method which aids in the study of atherosclerotic lesions is presented. This method augments, but does not replace, other approaches of investigation.

The basic tool is a gadget used by children to punch holes in paper or to make confetti. The puncher (Fig. 1) is used to obtain disks of aortic or other vascular tissue 6 mm. in diameter. The adventitia has to be stripped off before the puncher is used.

The disks can be used in many ways.

1. The thickness of normal and diseased tissue, of grossly different lesions, or of the center and edge of a single plaque can be measured with the aid of a caliper (Fig. 1). Since separation of layers of the morsel of vessel wall is easy, one also can measure the thickness of individual layers with the aid of a magnifying glass.

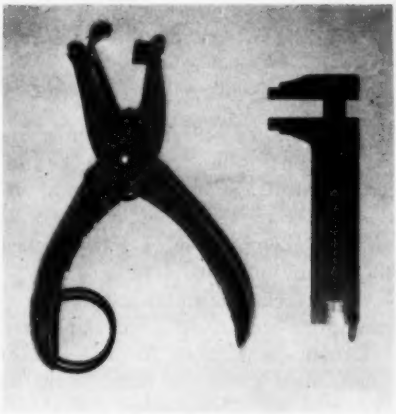

Fig. 1.—Puncher and caliper.

2. The disks (Fig. 2) can be weighed for correlation with thickness, volume, or chemical composition.

3. One can determine the volume of the tissue by dropping the disk into a graduated 10 ml. pipette which has been shortened by filing off the narrow mouthpiece, positioned vertically into a cork plate, and filled with water.

4. Using different solutions, one can determine the specific gravity of the tissue in a manner similar to measurement of the volume.

5. The tissue disk can be analyzed for organic (lipids, cholesterol, hexosamine, hydroxyproline, etc.) or inorganic (calcium, magnesium, etc.) components. Total ash can be determined.

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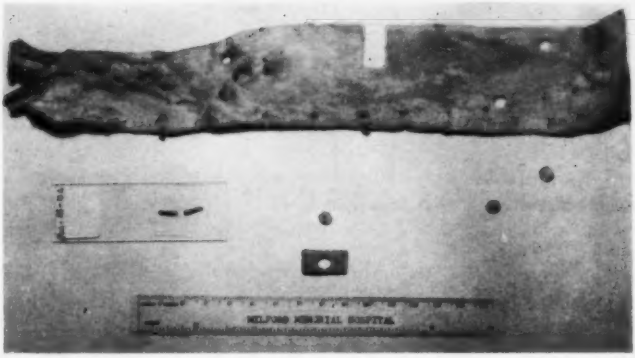

Fig. 2. — Aorta with punched-out holes and cut-out rectangle; punched-out disks and rectangular cut-out; slide with stained section of cut-out piece.

6. In lieu of chemical microanalysis (preceding pa), the tissue can be processed for histologic and histochemical studies. Microscopic study of cross sections also allows comparison of total thickness with that of individual layers.

7. To correlate chemical and microscopic findings, one can cut out a rectangular piece of vascular tissue about 20 mm. wide and 10 mm. high, using the punched-out hole as a center and cutting about 2 mm. above and below the hole and about 7 mm. to each side of the hole. Such a piece (Fig. 2) should be embedded on its edge and sections prepared at various levels. On the slide there will be two strips of tissue separated by a gap, corresponding to the punched-out disk (Fig. 2). The opposing ends of the strips will be lacerated, since they were traumatized by the puncher. Provided, however, that a lesion or, for that matter, a grossly normal sector exceeds 6 mm. in diameter, there will be no difficulty in comparing results of chemical analysis of the punched-out part with histochemical observations of the surrounding tissue.

Mutilation of the aorta by punched-out holes or cut-out rectangles is not severe, and the vessel can still be used for other purposes, such as gross staining or preservation as a museum specimen.

9 Kings Highway.

Diabetic Nephropathy

Sampling and Quantitative Evaluation of an Autopsy Population with Kimmelstiel-Wilson Lesions

STEPHEN M. SHEA, M.D., M.Sc.; STANLEY L. ROBBINS, M.D., and G. KENNETH MALLORY, M.D., Boston

Little that is precise is known of the relation of the lesion of nodular intercapillary glomerulosclerosis to that of renal hyaline arteriolosclerosis. The purpose of this paper is to investigate the possibility that a close quantitative relation exists between the severity of these two lesions when they coexist in cases of diabetes. If such a close relation should exist, it would not prove that the two lesions were causally related but would at least raise such a possibility. Contrary views have been expressed in the literature. Horn and Smetana [1] do not consider the Kimmelstiel-Wilson lesion to be necessarily associated with diabetes. They have cited in support of their point of view the suggestion of Newburger and Peters [2] that the primary lesion may be in the arterioles, and that the glomerular changes, and indeed the diabetes itself, may be secondary to the arteriolar lesion. Bell [3-5] in particular has commented upon the association in diabetes of severe nephrosclerosis with the Kimmelstiel-Wilson lesion. At one time this author [3] tended to the view that the glomerular lesion represented an extension of arteriolosclerosis into the glomerulus. More recently, Bell [5] has suggested that the severest cases of renal arteriolosclerosis occur only in diabetes and constitute examples of a specific diabetic lesion irrespective of the presence or absence of intercapillary glomerulosclerosis.

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Another aspect of renal disease in diabetes, the study of which has been based, up to the present, on relatively crude quantitative data, concerns the relation with duration of diabetes. This is the idea that the severity of intercapillary glomerulosclerosis is related to the duration of the diabetes. (Similarly, the relation between intercapillary glomerulosclerosis and renal arteriosclerosis has also been based on a subjective estimate of the severity of each process, as graded in terms of imaginary mental standards.) Such grades constitute an ordinal scale, which is at best a crude and highly subjective method of measurement. In the present paper we have set out to establish a more accurate, objective way of measuring the severity of intercapillary glomerulosclerosis and of benign nephrosclerosis, and then to establish more definitely whether there is indeed a relation between the severity of intercapillary glomerulosclerosis, on the one hand, and duration of diabetes, on the other, and, similarly, whether there is a relation between intercapillary glomerulosclerosis and benign nephrosclerosis.

Various authors [3,6-9] have commented on a rising incidence of intercapillary glomerulosclerosis, and possibly also increased severity with increasing duration of diabetes. Laipply,[10] however, disagrees. Perhaps the best established of these observations are the reports of a rising incidence with increasing duration of diabetes (Warren and LeCompte [8]; Rogers, Robbins, and Jeghers [9]). Much more questionable is the evidence demonstrating a relation between severity of intercapillary glomer-

ulosclerosis and duration of diabetes. For this reason, it is the latter type of relation with duration which we have chosen to study.

Two approaches are open in the study of relations of this kind. One can simply classify the cases according to arbitrary grades of severity of each lesion. This form of measurement is suitable for use with a large volume of data, but is open to the objection that there is no measure of objectivity, in terms either of consistency or of agreement among observers. Moreover, unless the most stringent precautions are taken, the allocation of grades to individual cases can be biased.

A more satisfactory scale of measurement is the "ordinal" scale, in which items are ranked according to the degree to which they possess a particular property.[11] This type of measurement is general enough for use with the most elusive biological data.

The ordinal ranking of histological lesions is made possible by a method involving the comparison of cases in pairs. The theory of the method of paired comparisons is due to Kendall and Babington Smith [12] and Kendall.[13]

An instance of its use in histopathology is given by Shea.[14] With this method one can rank slides in order of severity of a lesion and also find a measure of the objectivity with which the ranking is produced in terms of a coefficient of consistency (ζ) and of a coefficient of agreement among observers (u).

The interpretation of the results of a set of rankings of histological lesions is best expressed in terms of another coefficient (τ)—a coefficient of rank correlation (Kendall [13]). This is a measure of the similarity of two rankings and can be used to assess the degree of positive or negative correlation of severity of two lesions, or to establish their mutual independence.

Practical considerations confine the use of the method of paired comparisons to series of moderate length. In the present instance a sample of 20 cases was chosen. This involved the comparative evaluation of 190 pairs of cases for severity of intercapillary glomerulosclerosis and of renal arteriolosclerosis. These 20 cases were also ranked according to known duration of diabetes and insulin requirements. The object of the experiment was to estimate the correlation of the two lesions as to severity and to establish its significance. Evidence was also sought of a relation between the severity of each type of lesion and the duration and severity of the diabetes. From all these points of approach, it was hoped one could

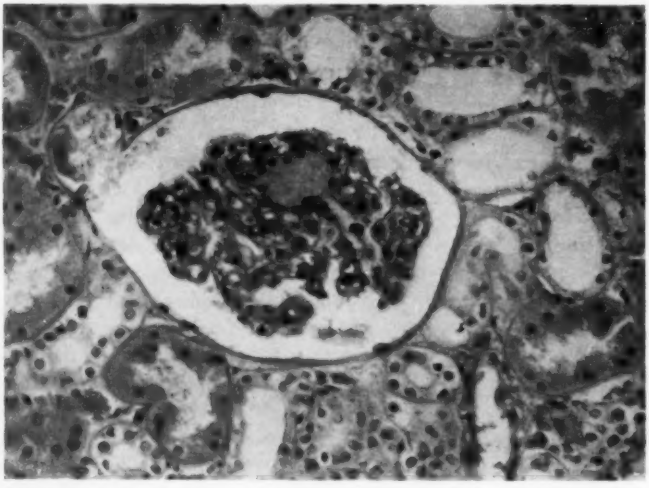

Fig. 1.—A nodular lesion in a glomerulus from Case B. Reduced to two-thirds of mag. × 100. Combined ICGS rank, 19½.

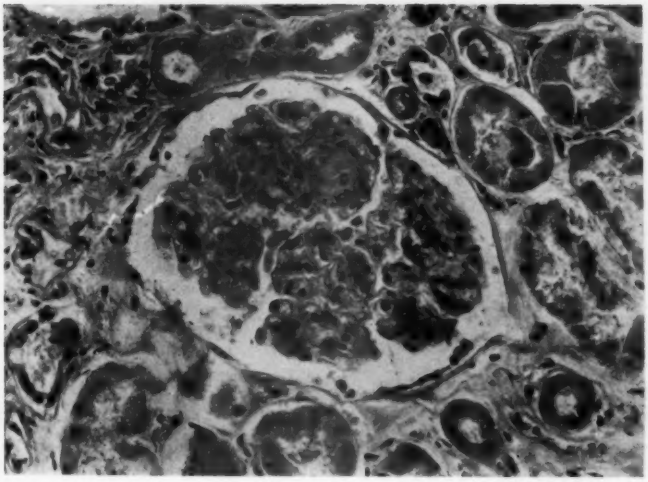

Fig. 2.—A nodular lesion in a glomerulus from Case N. Reduced to two-thirds of mag. × 400. Combined ICGS rank, 19½.

make some inferences concerning pathogenesis. Those who wish to apply the statistical methods adopted here are referred for particulars to the afore-mentioned authors.[12-14]

Methods

Criteria.—In order that all our cases may be accepted without question as valid, even the least severe, we have included only cases showing peripheral nodular glomerular lesions (cf. Allen[15]; Robbins, Rogers, and Wollenman[16]). Examples of mild cases are shown in Figures 1, 2, and 3. Cases showing only diffuse or axial lesions were excluded by this operational definition. The "diffuse" lesion was distinguished by Bell[8,4,17] from the nodular lesion. While it also may represent an outcome of the diabetic process, the diffuse lesion is difficult to distinguish from glomerular disease of other kinds.

In the severest cases the majority of the glomeruli showed advanced lesions (Fig. 4). Hyalinization of the afferent and efferent arterioles is illustrated in Figure 5.

As the method of ranking by comparisons involves an intuitive choice between the slides of individual pairs, it depends solely on a brief or prolonged examination of the slides of the two

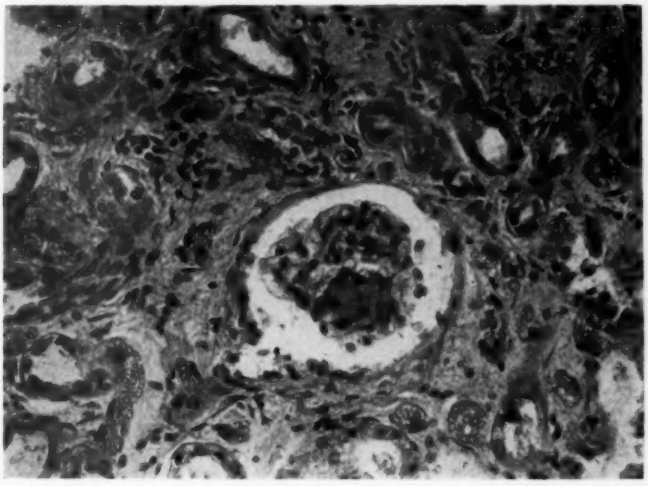

Fig. 3. — A nodular lesion in a glomerulus from Case N. Reduced to two-thirds of mag. × 400. Combined ICGS rank, 19½.

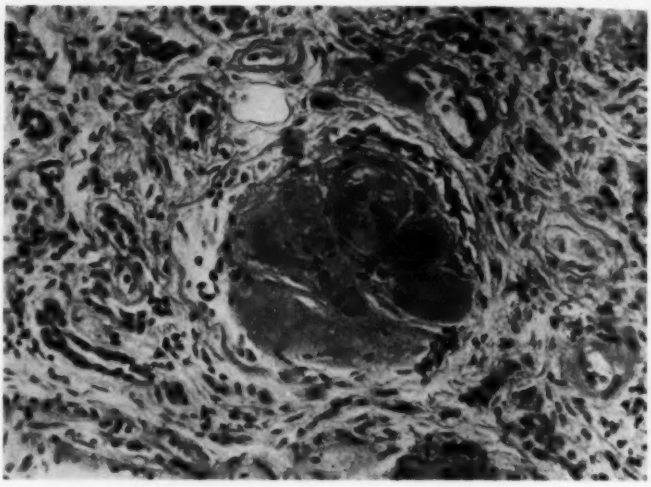

Fig. 4.—Severe intercapillary glomerulosclerosis in a glomerulus from Case T. Reduced to two-thirds of mag. × 400. ICGS rank, 1½; arteriolosclerosis rank, 4.

cases at the same session and replaces the effort to formulate explicit criteria of severity.

Selection of Material.—Eighty-three cases of diabetes mellitus that were autopsied at the Mallory Institute of Pathology during the period from 1953 through 1956 showed microscopic evidence of intercapillary (nodular) glomerulosclerosis. In 35 of these it was not possible to make with confidence a reasonable estimate both of the time of onset of the diabetes and of the maximum stabilized insulin requirements; these 35 cases were rejected, leaving 48 cases. From these, 20 were chosen by use of a recognized process of randomization. Routine autopsy sections of the kidneys, using tissue fixed in Zenker's fluid with acetic acid, and stained with hematoxylin and eosin or with Mallory's phloxine and methylene blue, were used for microscopic examination. All possible pairs of cases were studied to determine whether slides of one member or the other showed evidence of severer specific glomerular lesions, and were also studied according to the severity in each case of (juxtaglomerular) arteriolar hyalinization.

Clinically, the cases were arranged in order according to the known duration of the diabetes, and also according to the maximum stabilized insulin requirements. The maximum dose of insulin upon which a patient had been stabilized was taken as a measure of the severity of his diabetes.

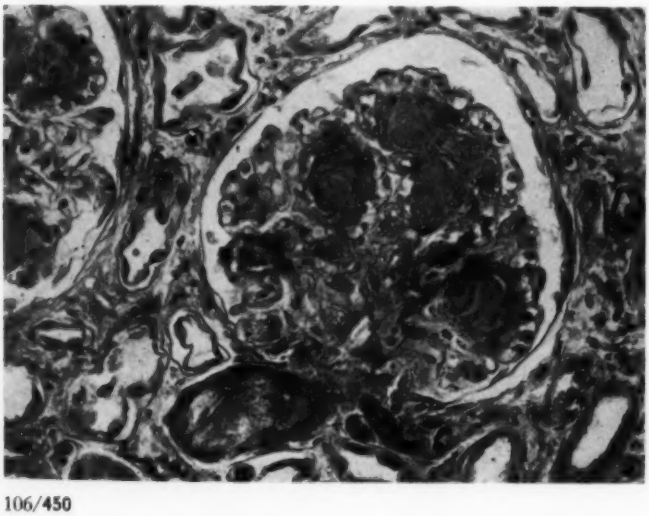

Fig. 5. — Intercapillary glomerulosclerosis with severe afferent and efferent arteriolar hyalinization, in a glomerulus from Case E. Reduced to two-thirds of mag. × 400. ICGS rank, 1½; arteriolosclerosis rank, 1.

TABLE 1.—*Twenty Cases of Intercapillary Glomerulosclerosis*

Case	A	B	C	D	E	F	G	H	I	J
Age	62	63	41	58	75	59	64	83	76	69
Sex	M	F	M	F	F	F	M	F	F	M
Duration of diabetes										
Years	16	9	15	15	7-8	5	13	32	11	7
Rank	3½	12	5½	5½	13	17	7	1	16	14
Insulin rank	5	7½	2	3	15	18½	10	13½	10	5
Case	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**
Age	66	83	57	70+	48	73	39	85	63	35
Sex	F	M	M	F	F	F	M	M	F	F
Duration of diabetes										
Years	12	10+	2+	2	6	5+	30	3+	12	16
Rank	8½	11	19	20	15	16	2	18	8½	5½
Insulin rank	5	16	18½	18½	13½	18½	10	12	1	7½

Results

Results are summarized in Tables 1, 2, 3, and 4. In Table 1 the patients are identified by a code letter, under which can be read the patient's age and sex, the duration of the diabetes, and the place in the ordinal

TABLE 2.—*Rankings of Twenty Cases of Intercapillary Glomerulosclerosis by Two Observers: Correlation of Severity of Intercapillary Glomerulosclerosis (ICGS) and Benign Nephrosclerosis (BNS)*

Rankings of Observer 1 *

Case		A	B	C	D	E	F	G	H	I	J
Rank order	ICGS	8	20	8	3	2	6	15½	10¾	14	10½
	BNS	6	20	15½	2½	1	15½	10	14	17	5
Case		**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**
Rank order	ICGS	12	5	17½	19	4	8	13	17½	15½	1
	BNS	12	2½	13	19	4	10	7½	18	10	7½

Rankings of Observer 2 †

Case		A	B	C	D	E	F	G	H	I	J
Rank order	ICGS	9½	19	5	7	1½	7	12	14½	17	12
	BNS	14	19½	7½	6	2	16	10	14	17	7½
Case		**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**
Rank order	ICGS	14½	7	9½	20	4	12	1½	17	17	3
	BNS	11½	4½	9	19½	2	11½	2	18	14	4½

Rankings Based on Comparisons of Both Observers ‡

Case		A	B	C	D	E	F	G	H	I	J
Rank order	ICGS	9	19½	6½	4	1½	6½	15	12	16	11
	BNS	8	20	13	4	1	16	9½	15	17	6
Case		**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**
Rank order	ICGS	13½	5	13½	19½	3	10	8	18	17	1½
	BNS	13	3	9½	19	2	11	5	18	13	7

* Coefficient of rank correlation, r, for these rankings is given by the formula $r_b=+0.571$. This shows the two rankings to be similar The probability that such similarity of rank order could occur by chance is less than $P=0.002$. The correlation is therefore highly significant.

Note: Rank numbers that recur or are fractional are due to ties.

† The coefficient of rank correlation, r, for these ranks is given by the formula $r_b=+0.765$. This shows the two rankings to be similar. The probability that such similarity of rank order could occur by chance is less than $P=0.002$. The correlation is therefore highly significant.

Note: Rank numbers that recur or are fractional are due to ties.

‡ The coefficient of rank correlation, r, for these rankings is given by the formula $r_b=+0.645$. This shows the two rankings to be similar. The probability that such similarity of rank order could occur by chance is less than $P=0.002$. The correlation is therefore highly significant.

Note: Rank numbers that recur or are fractional are due to ties.

TABLE 3.—*Coefficients of Consistency and Agreement*

	ICGS	BNS
Observer 1	Consistency ζ=0.879	Consistency ζ=0.785
Observer 2	Consistency ζ=0.881	Consistency ζ=0.797
	For Comparisons	For Comparisons
Agreement of Observers 1 and 2	u=+0.642	u=+0.632
	For Rankings	For Rankings
	r_b=+0.652	r_b=+0.687

TABLE 4.—*Severity of Intercapillary Glomerulosclerosis and of Benign Nephrosclerosis Correlated with Known Duration of Diabetes*

	ICGS-Duration Coefficient of Correlation	BNS-Duration Coefficient of Correlation
Rankings of Observer 1	r_b=+0.189 *	r_b=+0.216
Rankings of Observer 2	r_b=+0.241	r_b=+0.251
Rankings from comparisons of both observers	r_b=+0.225	r_b=+0.182

* Significant at P=0.02.

ranking according to that duration. The ranking according to insulin requirements is also given in Table 1. This depends upon the maximum dose of insulin upon which the patient was known to have been stabilized during his life. Thus, a rank of 1—the highest rank—would be given to the patient receiving the highest maintenance dose of insulin.

The rankings according to severity of renal lesions are given in Table 2. In this Table the rankings according to the severity of each type of renal lesion are given in juxtaposition, so that their similarity may be appreciated. This similarity is present in the results of each observer separately and in the combined results. More detailed comparisons of the rankings of the same lesion reveal the similarity of the rankings of the same lesion by different observers (Table 3).

It will be appreciated from a consideration of Table 2 and of Table 3 that there is a striking similarity of the rankings of cases for severity of glomerulosclerosis and of renal arteriolosclerosis. In fact, the rank correlation is as good as that which obtains between observers for a given lesion. The rank correlations of the two lesions with known duration are positive, and statistically significant, but rather weak (Table 4). No significant correlation was found to obtain between the severity of either lesion and insulin requirements.

Comment

The measure of agreement among observers may seem surprising, but very little evidence is available concerning acceptable values in material of this kind. In a previous paper (Shea [14]) a comparable measure of agreement was obtained among three observers, with another lesion. In this study the lesions were deliberately chosen to represent the whole range of severity normally encountered, and were not, as was the case with the renal lesions, chosen by randomization.

The results must be referred to the population studied, which is an autopsy population in a large city hospital, with patients who have lived in all degrees of control. Within this group the minimum amount of selection possible was exercised; no effort was made to choose cases with the most classic morphological lesions. In many instances microscopic examination was made difficult, for example, by intercurrent pyelonephritis, though not sufficiently so as to affect the consistency of the comparisons too seriously. Again, cases were not chosen because of the perfection of the clinical records; all that was required was reasonably reliable information concerning the onset of the diabetes and some data concerning the severity of the diabetic process, estimated in terms of insulin requirements, in the absence of infections, coma, or terminal illness. The further selection involved in choosing 20 cases was by randomization.

Thus, the cases are as randomly chosen as the protocol permits in a retrospective study of this kind, and the findings may be

expected to hold for the autopsy population at the Boston City Hospital and similar institutions. It seems reasonable to conclude that the evidence suggests that a strong positive correlation exists between the severity of intercapillary glomerulosclerosis and renal arteriolosclerosis, and that a positive correlation, which is much weaker, exists between the severity of both lesions and the duration of the diabetes.

Comment

The literature on intercapillary glomerulosclerosis has recently been reviewed by LeCompte.[18] An "exudative lesion" is among those which have been identified with the lesion originally described by Kimmelstiel and Wilson.[19] It is generally regarded as nonspecific and does not concern us in the present study. The diffuse lesion distinguished by Bell [3,4,17] has already been mentioned. Several of the earlier papers in which it was sought to associate the presence of intercapillary glomerulosclerosis with duration of diabetes accepted cases showing only diffuse lesions as instances of mild intercapillary glomerulosclerosis. This fact may account for contradictory reports. The balance of opinion has been in favor of the existence of a correlation between duration of diabetes and the incidence of intercapillary glomerulosclerosis among diabetics (as shown by Rogers, Robbins, and Jeghers,[9] who excluded cases showing only the diffuse lesion). The present study of the severity of intercapillary glomerulosclerosis was similarly confined to cases showing nodular lesions.

That nodular intercapillary glomerulosclerosis is practically if not quite pathognomonic of diabetes is shown by the rarity of reports of lesions in proved nondiabetics. Siegal and Allen [20] reported 1 case in an apparently nondiabetic person from a series of 100 hypertensive patients. Raphael and Lynch,[21] who have recently published a paper on the occurrence of Kimmelstiel-Wilson glomerulonephropathy in diseases other than diabetes mellitus, reported suggestive, but not diagnostic, lesions in 4 out of 46 cases of benign essential hypertension. Allen [15] reported small, nodular lesions in 5 out of 34 cases of chronic glomerulonephritis; Raphael and Lynch [21] found similar lesions in 1 out of 6 cases. In the rare cases in Rogers and Robbins' [22] series at the Mallory Institute of Pathology the disease turned out, on further inquiry, actually to have been diabetes. Those lesions described by Raphael and Lynch [21] in association with acute pancreatitis and with fatty livers seem to resemble lesions of the exudative type.

The low value of the positive correlation of severity of intercapillary glomerulosclerosis with duration of diabetes which we have found is of considerable theoretical interest. It would seem that it might be dismissed as being due merely to error in dating the onset of diabetes, which is undoubtedly difficult. However, a complete correlation with duration would require that the lesion progress at an identical rate in all these diabetics. This concept of a correlation of severity with duration is not the most reasonable, bringing to mind the image of a steady, uniform, temporal progression, i. e., a state of affairs in which all cases tend to become severer with time, but in which some cases progress rapidly and others slowly. That some such process does in fact occur is made clear by the much higher correlation of intercapillary glomerulosclerosis with the severity of the arteriolar lesion, a lesion itself also weakly correlated with duration. Nothing can be deduced from the data concerning variation in rates of progression of the lesions from time to time, or temporary reversals of the process.

The remarkably high value of the correlation of the severity of the nodular glomerular lesion with the severity of arteriolar hyalinization is in conformity with the reports of Bell [5] of a relation between the severity of arteriolar hyalinization and the presence of intercapillary glomerulosclerosis. In fact, it would appear that the correlation may be as good as the

limitations of the ranking procedure permit; that is, while a correlation of the order of +0.6 or +0.7 is far from perfect, much, if not all, of the defect may be due to inconsistencies in the comparisons, inconsistencies which may also account for much of the disagreement among observers.

The high value we have obtained for the correlation between the severity of intercapillary glomerulosclerosis and that of renal arteriolosclerosis does not lead us to the conclusion of Newburger and Peters [2] that the primary lesion is in the arterioles, the glomeruli being involved only secondarily. It is true that renal arteriolar hyalinization appears to be commoner among diabetics than is intercapillary glomerulosclerosis (87.3%, as against 41.8% among diabetic patients with gangrene—Bell [5]). However, while 16 of his 160 nondiabetic patients with gangrene showed some degree of renal arteriolar hyalinization, none showed intercapillary glomerulosclerosis. This sample is a small one, but the expected incidence of intercapillary glomerulosclerosis in the general population, if the condition can be caused by arteriolar changes in the absence of diabetes, can be estimated in another way. Moritz and Oldt [23] show that 97% of hypertensive patients coming to autopsy show some degree of renal arteriolosclerosis, whereas the lesion is present in 12% of the nonhypertensive patients. If 15% of the population is hypertensive (Moore [24]), then 24.5% of the autopsy population should show renal arteriolosclerosis. If renal arteriolosclerosis is expected to be found in one-fourth of autopsies, and if its relation to intercapillary glomerulosclerosis were as definite as is the case in diabetics, it might be expected to give rise to a substantial incidence of intercapillary glomerulosclerosis in the nondiabetic population—something in the order of 2% to 5%. This would appear to be contrary to general experience. For this reason, we do not feel that much, if any, part of the correlation we have demonstrated between the two lesions in diabetics is due solely to the prior presence of the arteriolar lesions.

On the contrary, it would appear more likely that some factor—a feature of the diabetic process necessary for the eventual formation of the pathognomonic nodular Kimmelstiel-Wilson lesions—must also accelerate the development of the less specific renal arteriolosclerosis.

Summary

A sample of an autopsy population showing the nodular glomerular lesions first associated with diabetes by Kimmelstiel and Wilson has been studied quantitatively by a nonparametric ranking method.

A marked correlation has been found to exist between the severity of the nodular lesions and that of renal arteriolar hyalinization. This is interpreted in terms of a causal relation, in which some aspect of the diabetic process, related to the genesis of the specific glomerular lesions, determines also the severity of the renal arteriolosclerosis.

There is a significant, if weakly positive, correlation between the severity of the nodular glomerular lesions and the known duration of the diabetes, and between the severity of the renal arteriolosclerosis and the known duration of diabetes.

No relation could be demonstrated between severity of intercapillary glomerulosclerosis or of renal arteriolar hyalinization and the severity of the diabetes as measured by insulin requirements.

We wish to thank Dr. Hugo Muench, Professor of Biostatistics, Harvard School of Public Health, who has reviewed the statistical calculations.

The photomicrographs are by Mr. Leo Goodman, of the Mallory Institute of Pathology.

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145 St. George St., Toronto (Dr. Shea).

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Myocardial Abscesses

A Study of Pathogenesis with Report of a Case

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Abscesses of the myocardium occur relatively infrequently, usually as part of an overwhelming pyemia involving multiple organs. In a few instances, however, they have been implicated in the spontaneous rupture of the heart. The following case is such an instance, which was unassociated with general pyemia because of the peculiar growth requirements of the infecting bacteria.

Report of Case

Clinical Summary.—A 67-year-old white woman was admitted to Hartford Hospital at 8:10 p. m., comatose, and died at 5:10 a. m. the next morning. Twelve years prior to admission, diabetes mellitus had been diagnosed, but she refused to observe diet or take insulin. During the past two years she had lost weight. Three months before admission, she started complaining of abdominal pain, and for the last three weeks she had loss of appetite, constipation, shortness of breath, and weakness. She was found unconscious in her room on the afternoon of admission.

She appeared unconscious, but she was able to withdraw from painful stimuli and to push away the examiner's hand. There was no apparent malnutrition. The skin was hot and dry. Pulse rate was 100; respirations 40, and deep but regular, with a questionable odor of acetone; blood pressure 104/60, and temperature 103 F (R). Pupils were round and equal, and reacted sluggishly. Optic discs showed no edema. The neck was supple. A few rales were heard in both lower lungs, but there was no dullness. The heart was not enlarged. Sounds were of poor quality; rhythm was normal, with a Grade 2 apical systolic murmur. The abdomen was flaccid, with a freely movable, firm mass in the right upper quadrant. Peristalsis was hypoactive. Deep tendon reflexes were inactive, but no lateralizing signs were noted.

Laboratory findings included a white cell count of 20,000 per cubic millimeter with 90% polymorphonuclear cells; plasma acetone 4+; urine sugar 4+, acetone 4+; blood sugar 574 mg. %; NPN 63 mg. %; CO_2 23 mEq. Blood was drawn for culture and a lumbar puncture performed, which returned clear spinal fluid.

In the next four hours the patient received 100 units of zinc insulin crystals and 500 cc. of 0.85% isotonic NaCl intravenously. The concentration of sugar and acetone in the urine fell to a trace. An hour before death the blood pressure fell to 60/50 and did not respond to intravenous administration of levarterenol (Levophed). She remained unconscious, and the temperature rose to 105 F (R) just before death.

Necropsy Findings.—Necropsy, excluding the brain, was performed five hours post mortem. The abnormal findings were mainly in the heart and gallbladder. The pericardium contained 100 cc. of fresh blood clot, and the surfaces of the pericardium were covered by a fibrinous exudate. The right ventricle appeared normal. The left ventricle contained several abscesses 3 to 10 mm. in diameter. Two of these were situated close to the epicardial surface and appeared fluctuant. One of

Fig. 1.—Posterolateral view of heart, showing multiple abscesses and perforation.

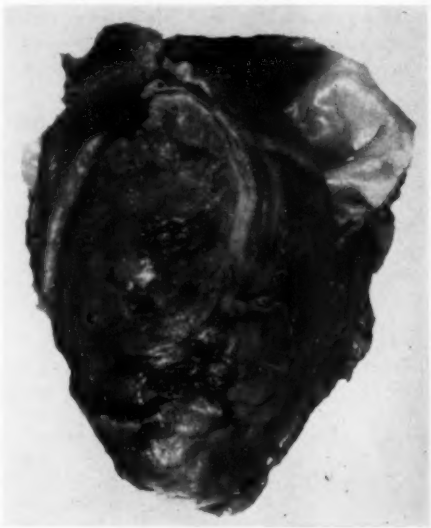

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Fig. 2.—Sagittal section, revealing abscesses of posterior apical septum.

these was the site of myocardial perforation, located in the posterior apical portion of the left ventricle near the septum. Upon opening the heart, other abscesses were seen in the apical septum and in the bases of several papillary muscles. The cardiac valves were normal, with delicate chordae tendineae and no endocardial vegetations. The coronary arteries showed severe calcific sclerosis, with an old occlusion of the right coronary vessel supplying the posterior apex and septum. There was a 1×3 cm. area of myocardial fibrosis in this

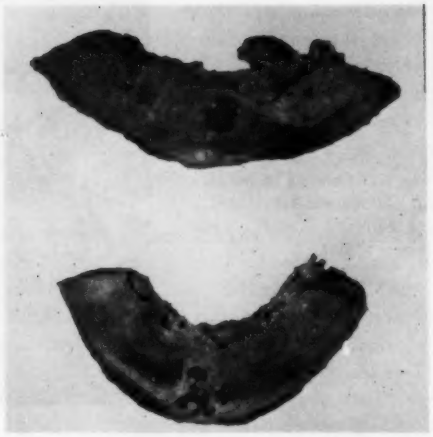

Fig. 3.—Cross sections of left ventricle, containing abscesses of myocardium and papillary muscle.

region, adjacent to the myocardial abscesses described above.

The gallbladder was distended to twice normal size with turbid, pinkish-white fluid. The wall was thickened by edema and fibrosis to 1.5 cm. The mucosa was hemorrhagic and partially sloughed. A solitary 2 cm. cholesterol stone was impacted in the gallbladder neck, but cystic and common bile ducts were patent.

The remainder of the viscera showed acute and chronic passive congestion and severe generalized arteriosclerosis. There were no other sites of suppuration.

Direct smears of the pus in the gallbladder and of the pericardial hematoma contained many plump, nonmotile, Gram-positive rods, occurring singly and in short chains. Capsules were present, and no spores were seen. Cultural behavior, in-

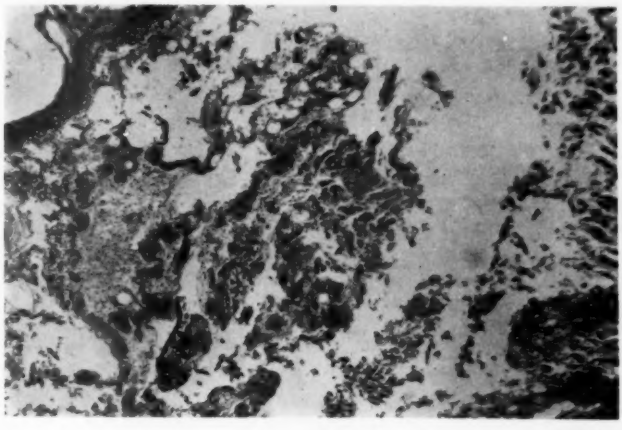

Fig. 4.—Point of rupture at the epicardial surface, showing necrotic muscle debris and collections of gas. Hematoxylin and eosin stain; × 100.

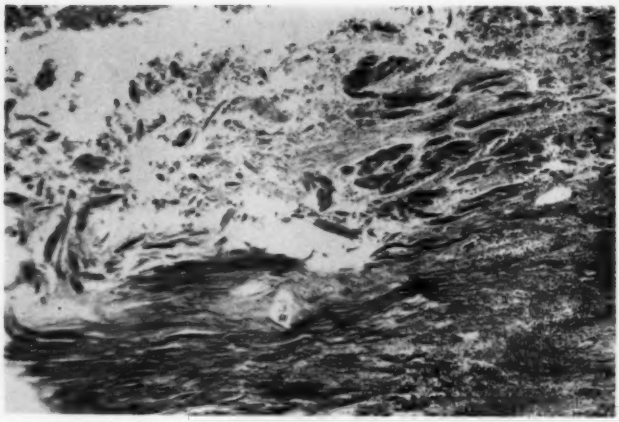

Fig. 5.—Acute inflammation and necrosis in an area of old myocardial scarring. Hematoxylin and eosin stain; × 100.

cluding characteristic stormy fermentation of milk, identified this organism as Clostridium perfringens (Bacillus welchii). A culture of small-bowel contents grew normal flora. Antemortem cultures of blood and spinal fluid were sterile.

The microscopic study of the tissues showed the myocardium involved by a suppurative process affecting all layers. The epicardial fat was infiltrated with polymorphonuclear leukocytes and overlaid by fibrin containing many red blood cells, leukocytes, and bacteria. At one point the epicardium was disrupted by hemorrhage in continuity with an abscess, containing necrotic muscle fibers, polymorphonuclear leukocytes, and myriads of short, plump bacilli. This abscess appeared to penetrate the ventricle adjacent to an area of old scarring, in which there was also acutely infarcted myocardium. Numerous small abscesses were seen in other sections of the apical left ventricle and septum. In all such lesions the muscle fragments were separated by empty spaces, sometimes cystic in appearance, which resulted from collections of gas produced by the bacteria. A section of the apical branch of the right coronary artery showed severe calcific atherosclerosis with a tiny eccentric lumen, probably a recanalization of an old occlusion.

The gallbladder mucosa was largely sloughed; a few tags remained mounted on persistent tunica propria. There was an acute inflammatory exudate of polymorphonuclear leukocytes involving all layers of the thickened, edematous wall. No Rokitansky-Aschoff sinuses were seen. The

Fig. 6.—Fragmented muscle fibers, leukocytes, and clouds of bacteria separated by gas bubbles. Hematoxylin and eosin stain; × 440.

muscularis contained many fibroblasts and macrophages, in addition to polymorphonuclear cells. Serosal blood vessels appeared normal.

The kidney showed numerous small cortical scars, with half the glomeruli hyalinized. All arteries and arterioles appeared markedly sclerotic, and a few glomeruli contained typical Kimmelstiel-Wilson lesions.

The remainder of the tissues were not abnormal for a person the age of the patient. No organ other than the myocardium and gallbladder showed any evidence of infection.

Comment

The pathogenesis of the myocardial abscesses in this case is unique. There is evidence in the myocardium of fresh infarction adjacent to the old myocardial scar. The abscesses had developed in these areas of muscle necrosis. The chronic cholecystitis with impacted cholesterol stone afforded the focus for the establishment of a Clostridium perfringens empyema. From this source blood-stream dissemination of these anaerobes found suitable conditions for growth in the dead heart muscle, with resulting abscess formation and eventual rupture of the myocardium.

Abscesses of the myocardium are rare, but there are enough cases in the medical literature to permit some conclusions concerning pathogenesis. Approximately 100 instances of abscess of the myocardium have been reported, chiefly in three series.[1-3]

In 50% of the cases the abscess of the myocardium occurred as part of an overwhelming sepsis. These cases have appeared primarily in the very young, the very old, and particularly those receiving mechlorethamine hydrochloride (nitrogen mustard) treatment for lymphoma and leukemia. The commonest organism has been Staphylococcus aureus, which accounted for 80% of the cases in one large series.[1] The myocardial lesions in these cases are usually not of primary clinical significance and frequently are microscopic in size.

A second category are those abscesses which develop by direct extension of subacute bacterial endocarditis, superimposed upon congenital or rheumatic valvular deformities. Such cases are usually reported as isolated cases, but in one large series of myocardial abscesses they formed 27.5% of all cases.[3]

A third group consists of those instances in which myocardial abscesses have occurred as the only metastatic site from a suppurative primary infection in some other organ. In Flaxman's[3] series of cases, 24% were attributed to acute osteomyelitis. The next commonest sources reported were pneumonia and cellulitis.

Forming a subgroup in this category are the very rare occurrences of abscess formation in acute myocardial infarcts. Three of these were attributed to concurrent bacterial pneumonia,[4] and a fourth case, to acute pyelonephritis.[5]

Perforation of the heart is described in each of the three categories, occurring in about 10% of reported cases. However, it is most conspicuous in the last group, where the added presence of acute myocardial infarction resulted in rupture in two out of five cases. Two additional perforations reported by Weiss and Wilkins[1] occurred in hearts with sclerotic coronary arteries containing focal thrombi near the abscesses, although no acute infarction was evident. This association of perforation with coronary artery disease is consistent with reviews of large series of spontaneous myocardial rupture in which acute infarction is the chief prerequisite to perforation.[6] It is noteworthy that the four patients in whom abscess with perforation occurred were elderly and debilitated, and two were diabetic. In such cases, in which bacterial infection is likely, prophylactic use of antibiotics may be indicated, in addition to conventional therapy of the cardiac lesion.

Summary

A case of myocardial abscesses with rupture and cardiac tamponade is reported.

The apparent source was Clostridium welchii empyema of the gallbladder, which spread via the blood stream to localize in a recent myocardial infarct.

The pertinent medical literature is reviewed and pathogenesis discussed.

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Lipid Deposits in Rats on Infarct-Producing Diets

An Electron Microscopic Study

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For several years diets containing cholesterol, thiouracil, and bile salts have been used by investigators to produce arteriosclerosis in rats,[1,2] but only in rare instances have the lesions thus produced been complicated by thromboses and infarcts. Recently we have demonstrated that the addition of large quantities of highly saturated fats (butter, Crisco, or lard) to the above regimen will result in the development of thrombi and infarcts in the hearts and kidneys of a significant percentage of rats (10% to 60%).[3,4] Addition of corn oil (a highly unsaturated fat) has seldom resulted in thrombosis and infarction.

Light-microscopic studies of these rats have been reported previously.[5] The thrombi and infarcts are similar to those seen in man except that thrombosis occurs with minimal alterations in the arterial wall, i. e., without intimal plaques. Abnormal deposits of fat are found in all organs, but particularly in the liver, spleen, kidneys, adrenals, arterial walls, and endocardium. These deposits, when stained with oil red O and examined by light microscopy, appear much the same regardless of the source and nature of the dietary fat. In contrast to the appearance of the fat with light microscopy, examination with the electron microscope reveals a wide variety of forms that the fat may take, perhaps reflecting differences in fatty acid content or protein linkage. The purpose of the present report is to describe and illustrate some of the morphologic characteristics, as observed with the electron microscope, of the abnormal deposits of fat in rats fed infarct-producing diets.

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Material and Methods

Details of the general procedures have been presented previously[3-5] and will be only summarized here. Male albino rats of the Wistar strain initially weighing approximately 100 gm. were housed in individual cages, weighed weekly, and fed variations of the diet presented in Table 1 ad libitum. A record was kept of their food intake. All animals that died during the course of the experiment, or that were killed, were autopsied and selected sections placed in cobalt nitrate-formalin for study by light microscopy.[4,5]

Ingredients of the Diets

	Per Cent by Wt.
Fat *	40.0
Cholesterol	5.0
Casein	20.0
Sucrose	21.5
Sodium cholate	2.0
Propylthiouracil	0.3
Celluflor †	5.0
Salt mixture ‡	4.0
Vitamin mixture §	2.0
Choline chloride	0.2

* Butter, butter oil, Crisco and corn oil were used as sources of fat.

† A cellulose flour.

‡ Wesson's modification of Osborne and Mendel salt mixture.[11]

§ Each kilogram of the mixture contained the following vitamins triturated in dextrose: vitamin A concentrate, 4.5 gm. (200,000 units/gm.); vitamin D concentrate, 0.25 gm. (400,000 units/gm.); *dl*-alpha tocopherol, 5.0 gm.; ascorbic acid, 45.0 gm.; inositol, 5.0 gm.; menadione, 2.25 gm.; *p*-aminobenzoic acid, 5.0 gm.; nicotonic acid, 4.5 gm.; riboflavin, 1.0 gm.; pyridoxine hydrochloride, 1.0 gm.; thiamine hydrochloride, 1.0 gm.; calcium pantothenate, 3.0 gm.; biotin, 0.02 gm.; folic acid 0.09 gm.

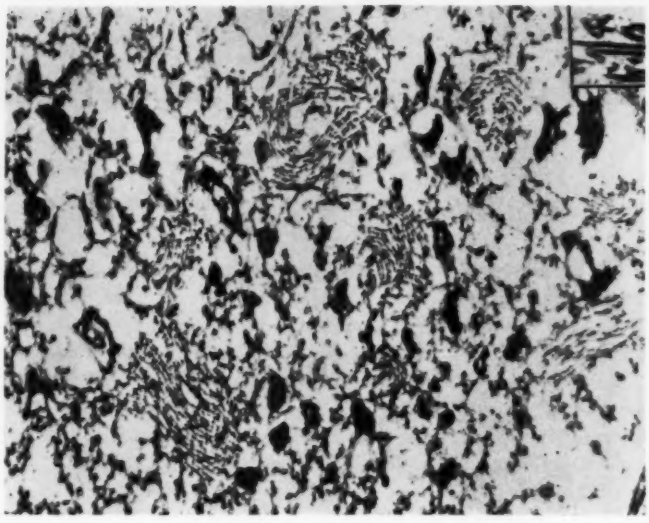

Fig. 1.—Blood plasma of rat fed butter and cholesterol. A small segment of vessel wall can be seen at lower right. The whorling, layered filamentous structures with an apparent periodicity of about 1,000 A. occasionally form an imperfect, but definite, "military" array. The identity of this material is not known, and it is only occasionally seen in the animals. The insert of collagen is cut from the same photograph for comparison of periodicities. The periodicity of the unknown material is not that which we usually associate with either fibrin or collagen. Black (osmophilic) aggregates of lipid are also present. × 18,000; reduced about ⅕.

Selected rats were killed over a period of one to four months from the beginning of the experiment to provide fresh tissue for electron microscopy. Fourteen rats received thiouracil, bile salts, cholesterol, casein, minerals, vitamins, and sucrose in the amounts shown in the accompanying Table. As a source of fat, four received whole butter, two butter oil, two Crisco, and six corn oil, all at the 40% level by weight. Blocks were taken from the heart, lung, kidney, adrenal, liver, aorta, and jejunum. These tissues were placed in Dalton's osmium tetroxide fixative* within 5 minutes of removal, and left for 1 to 2 hours, often with changes of the chrome-osmium solution every 15 minutes. The tissue was dehydrated with graded concentrations of ethanol and embedded in methacrylate. Thin sections were cut on a Porter-Blum microtome and examined in an RCA electron microscope, Model EMU 3B, at 1,000 to 18,000 diameters. Thicker sections from selected blocks were examined by phase microscopy.

Results

Abnormal deposits of osmophilic material, presumably lipids, were observed in all

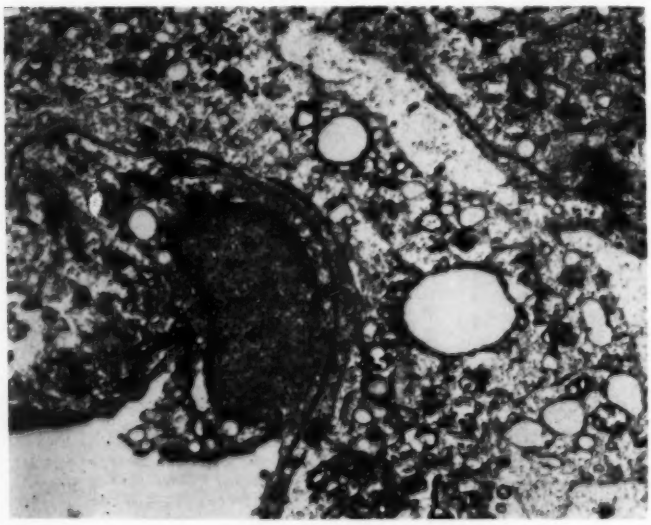

Fig. 2.—A pulmonary capillary traverses the field from lower right to upper left in a rat fed Crisco and cholesterol. An alveolar lining cell is present, and at left center collagen and elastic tissue lie between the alveolar and capillary walls. The capillary is almost filled by a macrophage containing many "ring" forms of lipid. × 12,000; reduced about ⅕.

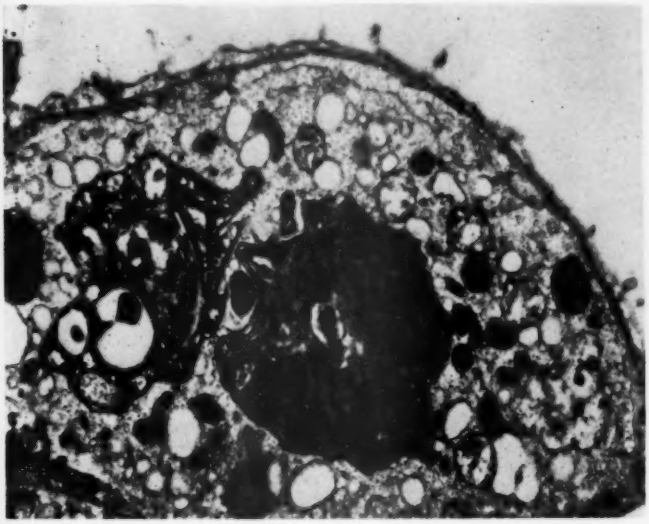

Fig. 3.—Rat fed Crisco and cholesterol. Large aggregates of lipid in a macrophage probably within the lumen of a pulmonary capillary. As in Figure 2, the cytoplasm of the macrophage appears to occlude the lumen. × 18,000; reduced about ⅙.

organs that were examined and in the blood (Figs. 1-10). These assumed a great variety of shapes, from small, rounded bodies to large, irregular masses. Many masses had clear centers, and these were particularly common in rats fed saturated fats, perhaps representing poor penetration of the osmium and dissolution of the unfixed centers of the fat globules during acetone flotation of sections. The degree of osmophilia (electron density) varied, some forms becoming jet-black, while others remained a light gray. Spherical, laminated forms, having some resemblance to myelin figures, were seen, particularly in rats fed corn oil, making it possible to distinguish tissues of these rats from those of rats fed the more saturated fats of Crisco and butter. No features have as yet been observed consistently in the rats fed butter

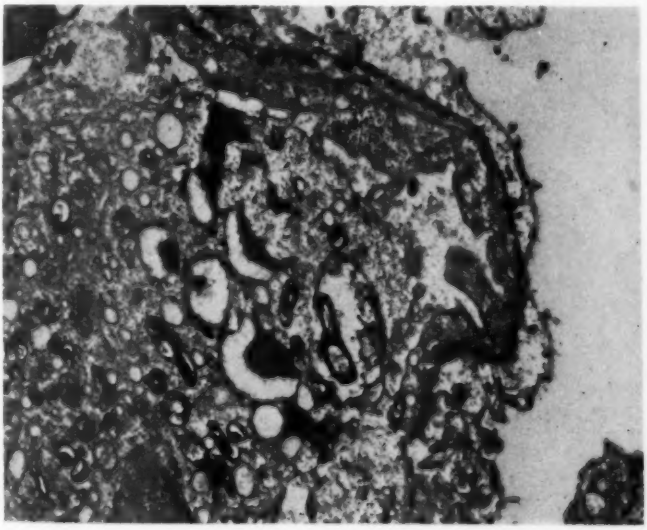

Fig. 4.—Pulmonary capillary and alveolar wall of a rat fed Crisco and cholesterol. The cytoplasm of the macrophage contains many lipid bodies, the small ones often having a central dark core with a surrounding clear space, suggesting layering of different components, but perhaps only artifact due to fixation. × 18,000; reduced about ⅕.

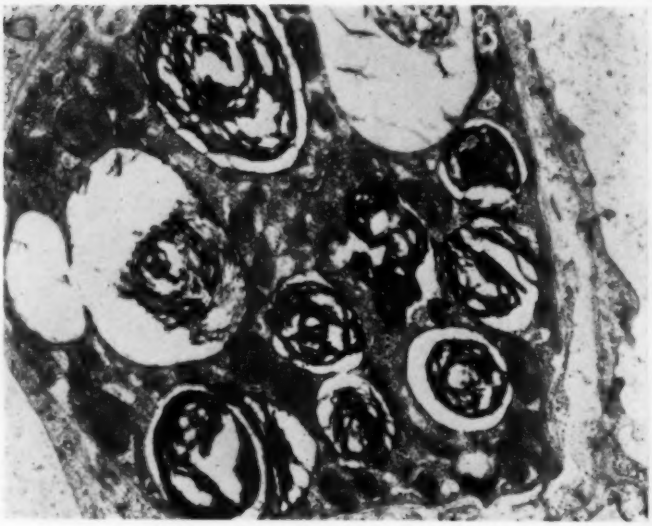

Fig. 5.—A macrophage in an interalveolar septum of a rat fed corn oil and cholesterol. These black, laminated, rounded lipid bodies were much less common in rats fed the more saturated fats. × 18,000; reduced about 1/5.

and Crisco that would serve to distinguish between the lipid deposits occurring in animals fed these two fatty substances.

Much of the osmophilic material in the blood and in tissues was in the cytoplasm of large macrophages, although some, in the blood, appeared to be extracellular (Fig. 1). In addition to the masses of free osmophilic material that are demonstrated in the plasma in Figure 1, we occasionally found whorled, layered filamentous structures (also seen in Figure 1). The nature of these structures is not known. They have a periodicity much greater than that we usually associate with fibrin and somewhat greater than the usual periodicity of collagen. Although variations in the periodicity of collagen have been described, it would be rather surprising to find collagen lying free in a vascular lumen.

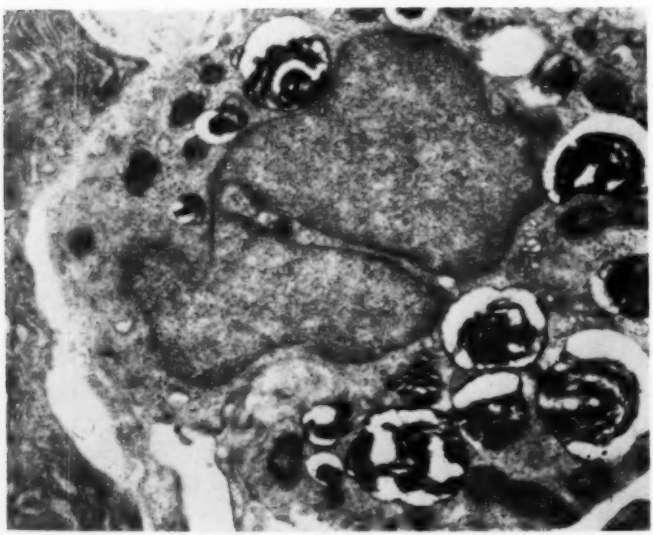

Fig. 6.—Another macrophage from a rat fed corn oil and cholesterol. The cell is lying in the interstitial space of an alveolar wall. On the extreme left is the cytoplasm of a plasma cell, with its prominent endoplasmic reticulum. The spherical lipid bodies are similar to those in Figure 5. × 18,000; reduced about 1/5.

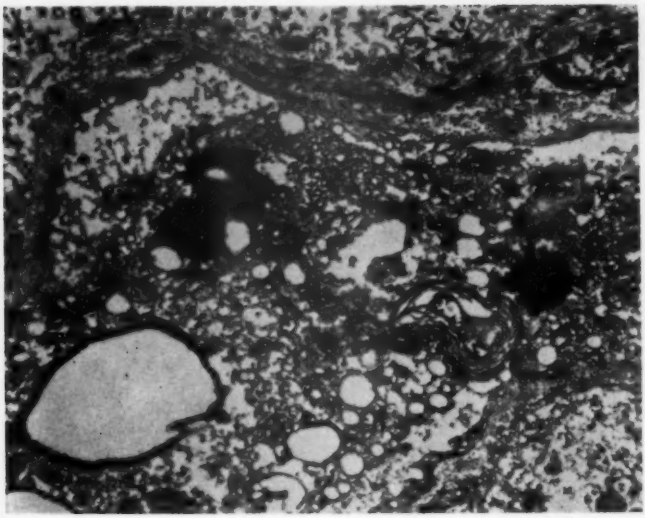

Fig. 7.—A small renal vessel from a rat fed butter and cholesterol. Solid lipid bodies, "ring" forms with clear centers, and gray, laminated forms are present in the cytoplasm of the macrophage that fills the vascular channel. × 11,000; reduced about 1/5.

Abnormalities other than those associated with the deposits of lipid were not observed. Surprisingly little osmophilic material was demonstrated in endothelial cells. No changes of the endothelial surfaces were found to account for thrombosis, although we were not fortunate enough to find an actual thrombus in the tiny blocks taken for electron microscopy.

Comment

The significance of the variety of forms of osmophilic material that were observed is not known. It is possible that each represents a different lipid or lipoprotein complex. The fact that certain forms appeared to be commoner in rats fed corn oil than in those fed saturated fats at least suggests some relation between form and chemical

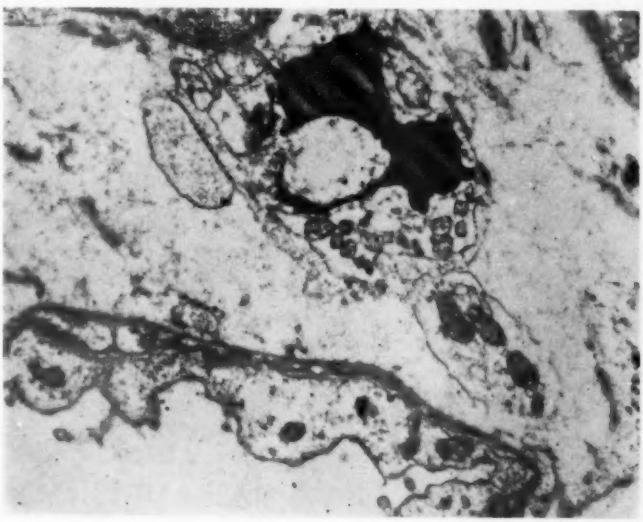

Fig. 8.—Renal medulla of a rat fed butter and cholesterol. The cytoplasm of a connective tissue cell at the top center contains a large aggregate of lipid. Below is a portion of the thin limb of a loop of Henle. × 18,000; reduced about 1/5.

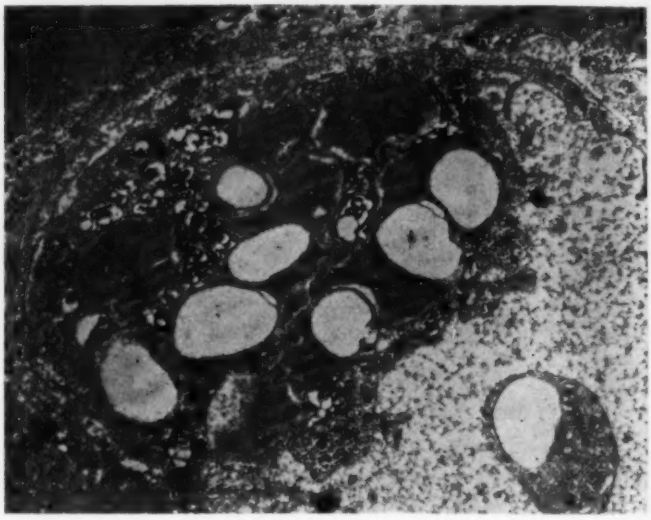

Fig. 9.—A macrophage appears to be clinging to a capillary wall in the myocardium of a rat fed butter and cholesterol. Small, dark clumps of lipid are present in the macrophage, but most of its cytoplasm is occupied by lightly staining granular material, possibly lipoprotein. × 12,000; reduced about ⅙.

content. However, many more studies with simpler systems must be carried out before such relationships are established. Electron microscopic studies of certain phospholipids in the leech have been made and identifiable concentric structures containing phospholipid demonstrated,[8] but more such studies are needed.

It is apparent that the morphologic features of the lipids observed in fixed specimen are not necessarily those that would be seen during life. However, as Deane has observed,[9] it is probable that even the alterations due to fixation are governed by the nature of the lipid. As purified fatty acids become available for feeding experiments in animals, the identification of complexes of these individual fatty acids by their electron microscopic pattern may be possible.

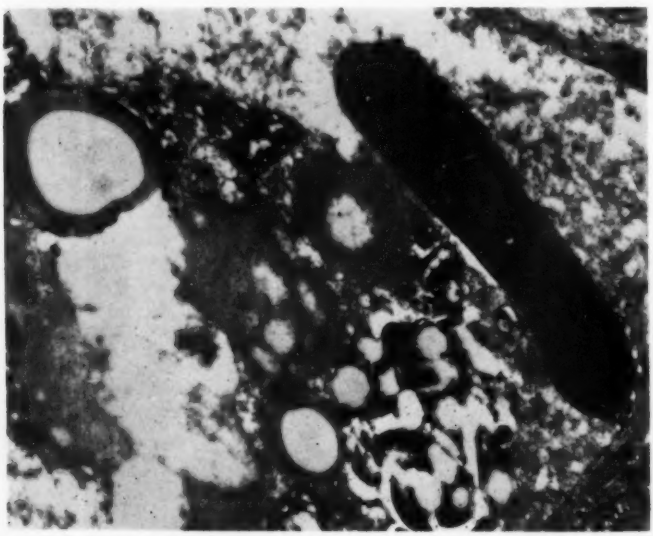

Fig. 10.—A macrophage within a capillary lumen in the myocardium of a rat fed butter and cholesterol. A red blood cell lies in the upper right quadrant of the photograph. At the upper left, deeply osmophilic lipid coats the outer surface of a gray, granular mass of material similar to the aggregates seen in Figure 9. × 19,000; reduced about ⅕.

The electron microscopic observations made thus far do not tell us why these animals develop thromboses and infarcts. However, the sparsity of endothelial-cell changes visible by electron microscopy, coupled with the failure to demonstrate definite plaques grossly or by light microscopy, suggests that something more than a local lesion of the arterial wall at the site of thrombosis is involved. It is possible that the high-fat diet has resulted in some alteration in the clotting or fibrinolytic mechanism of the blood, as has been demonstrated in other situations.[10]

By both electron and light microscopy many capillaries seem to be plugged completely with lipid or lipid-laden macrophages. Such plugs could serve as foci for beginning thrombus formation with retrograde extension. However, thus far we have not identified fibrin intermingled with the fat in the capillaries, nor have we found multiple small thrombi and microscopic infarcts. Instead, particularly in the heart, thrombi and infarcts have usually been single and of macroscopic size, at times involving almost the entire left ventricle.

Summary

In rats, diets containing cholesterol, thiouracil, bile salts, and certain saturated fats have been shown to result in the production of coronary and renal arterial thrombi with infarcts. These diets result in marked hyperlipemia with extensive deposits of fat in various tissues. Examination of the tissue and blood of these rats with the electron microscope reveals a multiplicity of forms that the fat may take. Certain forms appeared to be commoner in rats whose diets included corn oil than in those whose diet included saturated fats, suggesting that differences in form may reflect in part differences in chemical content. Fixation artifacts undoubtedly contribute to the electron-microscopic appearance to an unknown extent, necessitating caution in interpretation. But it is probable that even the alterations due to fixation are governed by the nature of the lipid.

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Papillomatosis of Trachea and Lung

Report of a Case

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In children papilloma is the commonest benign tumor[1] of the larynx. Generally this polypoid lesion is singular, but multiple papillomata of the larynx are not unusual. These tumors are treated most commonly by surgical extirpation, but the rate of local recurrence is high. On rare occasions, similar lesions may be present or subsequently appear in the trachea, major bronchi, or even the small pulmonary bronchioles.[2-5,8,9] Buffmire and associates[9] reported a case of laryngeal papillomatosis treated for many years which eventually developed into squamous-cell carcinoma. Later a cystic lesion appeared in the lower lobe of the left lung, which, on resection, also proved to be a papillary squamous-cell cancer.

In contrast, tracheal papilloma occurs only one-hundredth[7] as frequently as laryngeal papilloma. Since we have been unable to find any previous report of a tracheal papilloma[7,10,11] associated with similar pulmonary lesions, the present case is presented.

Report of Case

An 8-year-old white boy was admitted to the Albany Hospital on July 19, 1955, because of "intractable asthma." In the past he had had occasional episodes of acute bronchitis, which precipitated asthmatic attacks. However, during the previous three years his "asthma" had become severer and resistant to all medication. In addition, the boy had had progressive loss of appetite and had lost between 15 and 20 lb. in weight. Recently he tired more easily, was less inclined to play, and expressed an increased desire to sleep. On admission the child was using four or five pillows for respiratory comfort in bed. On physical examination the boy appeared dehydrated, poorly nourished, and listless, and had obvious respiratory difficulty. His maximum respiratory distress appeared to be in inspiration, and on expiration no wheezes were heard. These findings suggested an organic obstruction of the airway. Careful examination failed to reveal any laryngeal pathology. He was taken to the operating room for bronchoscopy. Ether and nitrous oxide were used for general anesthesia, and during induction his respirations became very labored and virtually ceased. Under direct observation, an endotracheal tube was inserted into the larynx, but respirations did not seem to improve. In spite of supportive measures, including bag breathing with oxygen, the child died.

At necropsy, the tongue, larynx, and tracheobronchial tree were removed en bloc. On gross examination a firm white papillary tumor (Fig. 1),

Fig. 1.—Solitary tracheal papilloma and cystic papillary lesion in upper lobe of right lung.

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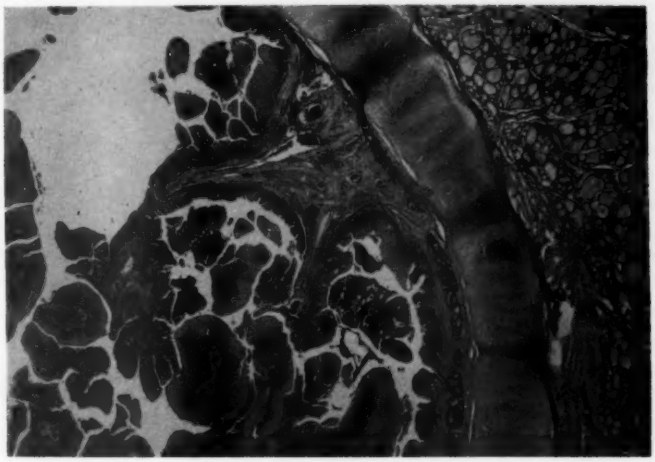

Fig. 2.—Tracheal papilloma. H & E; reduced to 63% of mag. × 20.

arising on the right lateral wall of the trachea, was found. This neoplasm measured 1.7 cm. in greatest diameter. The base of the lesion overlaid the first four tracheal rings and measured 0.9 cm. in width. The tumor almost filled the lumen of the trachea. In the lungs there were two yellowish, firm cystic lesions, each measuring 1.2 cm. in diameter, which on opening presented multiple small papillary excrescences. One lesion was in the posterior segment of the upper lobe of the right lung, and the other lay in the posterior basal segment of the lower lobe of the left lung. Careful dissection of the bronchi suggested that these cystic lesions were in continuity. However, one could not enter these pulmonary lesions directly because of the bronchial stenosis immediately proximal to these lesions.

In general the pulmonary tissues were congested and emphysematous, and in the bases foci of atelectasis were present.

The remainder of the organs showed only acute congestion and focal petechiae.

Sections of the primary tracheal tumor revealed a benign squamous-cell papilloma (Fig. 2). Each fold of the papillomatous mass which projected into the tracheal lumen was composed of a slender vascular connective-tissue core, which supported a thick layer of stratified squamous epithelium. The bulk of the epithelium was composed of prickle cells, and near the periphery hyaline-droplet and hydropic de-

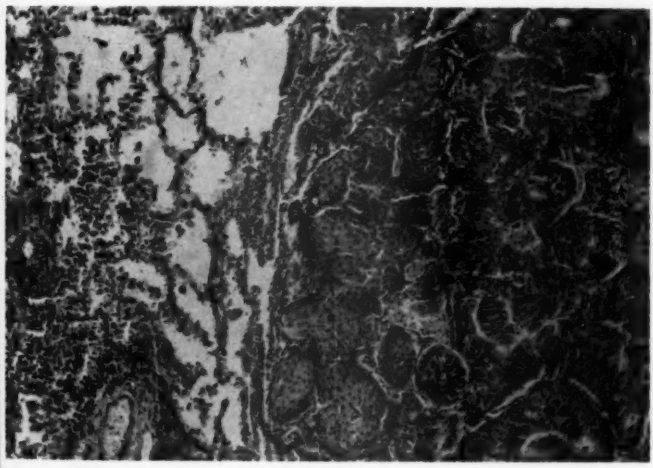

Fig. 3. — Solid portion of pulmonary lesion, filling alveoli and extending through pores of Kohn without alveolar-wall destruction. H & E; reduced to 63% of mag. × 120.

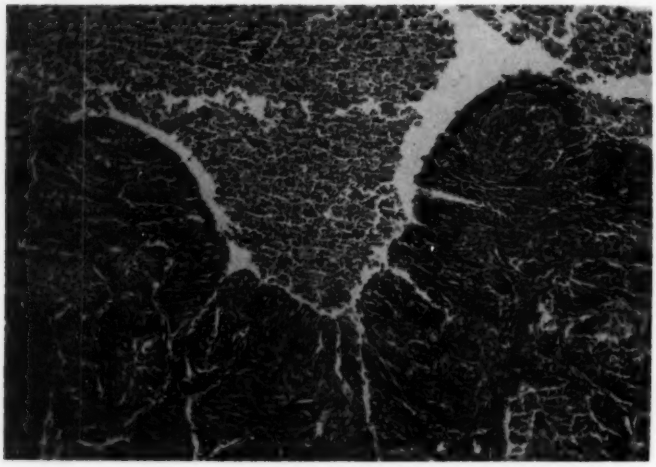

Fig. 4.—Cystic portion of pulmonary lesion, lined by single layer of columnar epithelium. H & E; reduced to 63% of mag. × 120.

generation of these cells were seen. There were a few mitoses in the basal cells and focal loss of polarity. There was no suggestion of invasion. The underlying submucosa was congested and chronically inflamed. In the adjacent tracheal mucosa there were several small zones of squamous metaplasia. Elsewhere the trachea was not unusual.

On microscopic examination the two yellowish cystic lesions of the lung appeared to be definitely related to terminal bronchioles. Metaplastic squamous epithelium could be seen arising from the terminal bronchiolar epithelium, extending into the alveolar sac and alveoli. This proliferating epithelium, utilizing the alveolar wall for support and nourishment, would fill the alveolus (Fig. 3) and spread to adjacent alveoli through the pores of Kohn. When the epithelium merely lined the alveolus, rather than fill it, the innermost cells usually showed marked keratinization. Rarely a single layer of tall columnar cells, resembling bronchiolar epithelium, covered the squamous elements (Fig. 4). The alveolar walls remained intact, and showed only mild infiltration by a few chronic inflammatory cells.

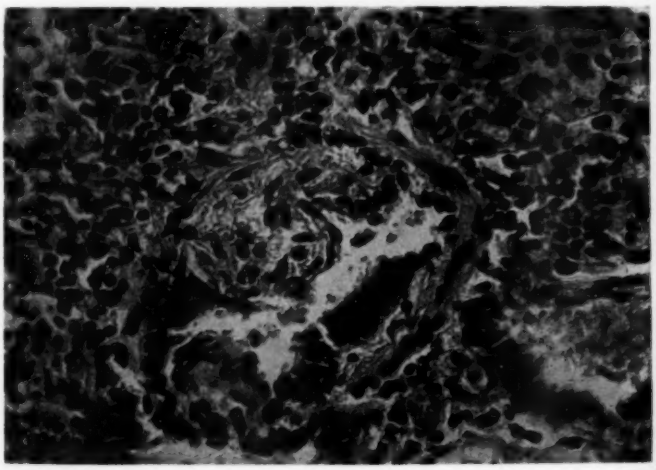

Fig. 5. — Obliterating bronchiolitis. H & E; reduced to 63% of mag. × 615.

The major bronchi frequently contained inflammatory exudate, and the bronchiolar walls were thickened, owing to hypertrophy of muscle and infiltration by both acute and chronic inflammatory cells. In places the cellular infiltrate extended into the adjacent alveolar walls, and there was lymphoid-nodule formation. Although careful search was made, no inclusion bodies were found in the respiratory epithelium. In places the terminal bronchioles were dilated, and their lining epithelium was focally ulcerated. Beneath these regions of ulceration there was proliferation of granulation tissue (Fig. 5). Not infrequently this granulation tissue bulged into the lumen of the bronchioles or the alveolar sac. Elsewhere the bronchiolar wall was swollen and infiltrated by numbers of chronic inflammatory cells. Some of the alveoli contained a few macrophages, with abundant pale, vacuolated cytoplasm.

In general the vesicular tissues showed small foci of atelectasis and diffuse areas of compensatory emphysema.

Comment

Different investigators have suggested different interpretations for the origin of lesions similar to the papillomata which occur or recur in sites distal to the primary tumor. In 1922 Ullmann [12] produced similar lesions on human skin and on the mucosal surface of dogs by injection of a cell-free filtrate prepared from the laryngeal papillomata of a child. Thus, many believed that laryngeal papillomata and related lesions were not truly neoplasms but, rather, were localized foci of epithelial hyperplasia secondary to virus inoculation. In addition, the tendency for these tumors to disappear at puberty spontaneously invited discussions of their relation to hormones. Hitz and Oesterlin [6] suggested that the distal recurrences actually resulted from endobronchial dissemination and implantation of a portion of the primary tumor separated by trauma. Buffmire and associates [9] felt that these lesions represented a multicentric origin of similar tumors, not unlike papillary neoplasms in the urinary tract. In regions of terminal bronchiolitis zones of squamous metaplasia were not found. Robbins and Sniffen [14] and Fienberg [13] have observed pneumonitis of the cholesterol type with bronchiolar changes, similar to the pulmonary changes of this case, without obstruction of the major bronchi. Furthermore, Fienberg has suggested that the obliterating bronchiolitis may represent a hypersensitivity phenomenon. In contrast, where metaplastic squamous epithelium arose from terminal bronchiolar epithelium, there was minimal associated inflammatory-cell reaction. These findings do not support the mechanism of endobronchial spread and implantation of a portion of the primary tumor. Unfortunately, we did not consider virus culture. However, in the tumor cells and in the respiratory epithelium no inclusion bodies were observed. Because of multiple foci of squamous metaplasia of the tracheal epithelium adjacent to the tracheal papilloma and the direct observation of squamous metaplasia from terminal bronchiolar epithelium, we favor the concept that multiple papillary lesions of the respiratory tract represent a multicentric origin of similar tumors, which may be of virus etiology.

Summary

A case of primary tracheal papilloma with associated similar lesions in the lungs is presented. The methods of dissemination of the primary tumor to the lungs are discussed.

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News and Comment

PERSONAL

National Institutes of Health Fellowship Committee Appointments.—Dr. Howard C. Hopps, chairman of the pathology department at the University of Texas, and Dr. John B. Graham, professor of pathology at the University of North Carolina, have been appointed as members of the selection committee for the senior research fellowship program of the National Institutes of Health, Bethesda, Md.

Col. Frank M. Townsend Appointed Director of AFIP.—Col. Frank M. Townsend, of the U.S. Air Force, has been appointed director of the Armed Forces Institute of Pathology, Washington, D.C.

Dr. V. A. Stembridge Receives Legion of Merit.—Dr. V. A. Stembridge, associate professor of pathology at University of Texas Southwestern Medical School, Dallas, Texas, has received the Legion of Merit of the Air Force in recognition of his meritorious service as chief of the aviation pathology section of the Armed Forces Institute of Pathology.

Dr. Shields Warren's Appointment.—Dr. Shields Warren, of Boston, has been made co-chairman of the Boston University Development Council.

Books

Progress in Hematology. Vol. II. L. M. Tocantins, M.D., Editor. Price, $9.75. Pp. 290. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1959.

This is a collection of thirteen reviews of hematologic topics. The subjects are well chosen, several containing information which has not often been brought together in one review. For example, Stuart C. Finch ably reviews the Transmission of Leukemia, discussing both animal and human experiments; Serotonin: Hematologic Aspects is well reviewed by Zucker. Other reviews which appear range in scope from a scholarly discussion, with over 350 references, of the Role of Physical and Chemical Factors in the Sickling Phenomenon by Harris, to a brief practical paper on the Correction of the Hemorrhagic Complications of Esophageal Varices. Unfortunately, there are a number of inaccuracies in the extensive tables of the Harris paper. The reviews are well written and authoritative. This book may be recommended to anyone with an interest in hematologic subjects. If, however, the reader expects to be kept abreast of all recent significant developments in hematology, he will be disappointed, for some developments in hematology which are very important have been left out. Some important topics not included in this volume are studies of haptoglobins and studies of red cell metabolism particularly related to drug-induced hemolytic anemia, galactosemia, and methemoglobinemia. It is hoped that subsequent volumes in this fine series will cover these subjects also.

Tumors and Tumorous Conditions of the Bones and Joints. By Henry L. Jaffe, M.D. Price, $18.50. Pp. 629, with 701 illustrations. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1958.

Many pathologists feel some confusion with the problem of orthopedic pathology. Some of this confusion may be traced to the lack of agreement among those who write in this field. Men in several specialties have contributed significantly to the literature of bone tumors. Outstanding work has been done by general surgeons, by orthopedic surgeons, and by radiologists, in addition to the contributions of pathologists. While such cooperative efforts are most rewarding in the investigation of disease processes, they run the risk of producing a conflict in concepts and terminology. It is this confusion in terminology, which is particularly prevalent in orthopedic pathology, that often causes confusion in the literature. Thus, it is fortunate that Dr. Jaffe, who has made immense contributions to the subject of orthopedic pathology, has seen fit to write, in a single integrated volume, about bone tumors.

After an introductory chapter on classification and diagnosis of bone tumors, the volume covers in a concise, orderly fashion the various bone tumors, starting with those which are commonly benign and continuing with malignant tumors. The final third of the book describes some of the more unusual bone problems which exist, such as the relationships of preexisting bone disease and radiation to tumors, invasion from soft-tissue tumors, synovial tumors, and metastatic tumors. Each of the significant bone tumors is considered in a chapter that includes clinical features, radiological appearance, and gross and microscopic pattern. Differential diagnoses are well covered in each chapter, and usually a brief outline of treatment is included. The chapters are profusely illustrated, with excellent clinical, roentgenographic, and pathologic pictures.

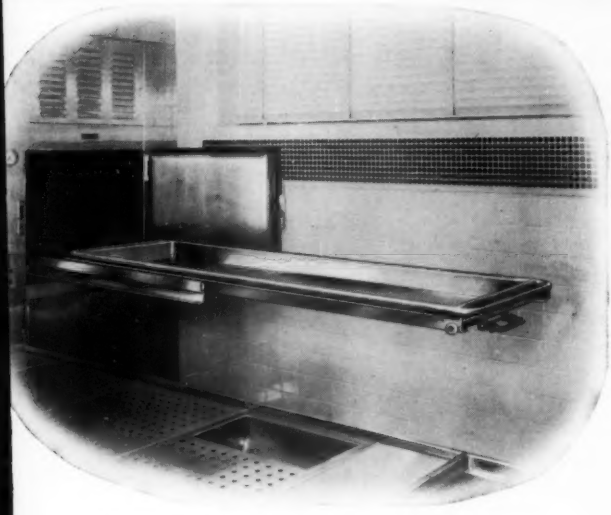
In a professional lifetime devoted primarily to the study of orthopedic pathology, Dr. Jaffe has understandably developed some topics of special interest. These interests are reflected in this book, where perhaps the chapters on fibrous cortical defect, osteoid-osteoma, and fibrous dysplasia are more emphatic than the clinical frequency of the lesions warrants. However, the reader is the richer for Dr. Jaffe's inclusion of his own material, as well as that of others, in these particular sections. Elsewhere through the book there is fine balance in the space and emphasis devoted to the various tumors. While the book will find its greatest value with pathologists, it also has much to offer others, such as surgeons, orthopedic surgeons, and radiologists, who are interested in the problems of bone tumors. It will prove to be a standard reference in its field.

Parasitology (Protozoology and Helminthology). Second Edition. By K. D. Chatterjee, M.D. Price, 17.50 Rs. Pp. 188, with 94 illustrations (16 colored plates). S. Bhattacharya & Co., 49, Dharamtallah St., Calcutta 13, India, 1959.

This book is certainly adequate to the fulfillment of the stated purpose of its author. It is designed to supply the medical student with the basic factual material that he requires in parasitology. It is a well-illustrated, systematic summary, rather than an extensive reference work, and its scope is limited to medical parasitology. The book should be useful to teachers, particularly to teachers of elementary medical parasitology.

The author has followed a trend which has been evident in medical parasitology for some years. This trend is away from the classical study of parasitology, which regards medical parasitology as one aspect of the general subject, and indeed an incident of not very great importance. The classical approach has much to recommend it in teaching parasitologists, but it is of less value in teaching medical students. Thus, the trend toward reduced complexity, with medical aspects of parasitology in sharper focus, is continued.

The author of the book is also the author of a much more comprehensive work in parasitology, which is not replaced by the current work. In fact, users of "Parasitology" should have more comprehensive works available as reference material.



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